ANTIOXIDANT POTENTIAL OF *Malphigia glabra* (ACEROLA) BERRIES IN RATS

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis, entitled **"ANTIOXIDANT POTENTIAL OF *Malphigia glabra* (ACEROLA) BERRIES IN RATS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Introduction

1. INTRODUCTION

Oxidative stress is a harmful condition of increased oxidant production in animal cells characterized by an excess release of free radicals, a decrease in antioxidant levels, or both, leading to cellular degeneration. All living cells are exposed to oxidative stress. Various stress related conditions such as chronic disease states, ageing, toxin exposure, physical injury, exposure to UV radiation and foods rich in saturated fatty acids and carbohydrates can enhance this oxidative process and cause cell damage, thereby reducing the quality and quantity of production in animals. Oxidative processes that take place in the body pave way to energy production, which is a continuous process. As a result of this oxidation, reactive oxygen species (ROS), such as free radicals like hydroxyl ions, nitric acid and superoxide ions or the non-radicals like hydrogen peroxides and lipid peroxides are produced. Reactive oxygen species interact with other molecules within the cells and cause oxidative damage.

To defend against oxidative damage, the body produces several antioxidants which neutralise or "clear up" free radicals that harm the cells. Sometimes internal production of antioxidants may not be enough to neutralize all the free radicals. Therefore, intake of antioxidants particularly those naturally available in foods, beverages and plants will be beneficial in reducing oxidative damage. Citrus fruits, apples, grapes, dark cherries and berries, onions, tomatoes, carrots, greens, red wine and black tea are some of the major sources of dietary antioxidants. *Malphigia glabra* (Acerola) berry is a juicy fruit, sour to taste and has a sweet, fruity flavour. It is widely available in Kerala and is a potent antioxidant. This fruit and its derivatives are principally known for their high vitamin C content and other nutrients such as flavonoids and anthocyanins which form part of polyphenolic compounds. Vitamin C, flavonoids and anthocyanins are well documented for their antioxidant property. The Acerola fruit has a vitamin C content of 11-14mg/g (Filgueiras *et al.,* 2003), which is 65 times greater than that in orange. It is also known to have high levels of vitamin A and

B as well as minerals such as calcium, iron, phosphorus, magnesium and potassium.

Liver is the major organ responsible for the detoxification of drugs and toxic chemicals. It is the primary target for many toxic materials and is very vulnerable to oxidative stress. Hepatotoxicity results in grave consequence and hence there is an ever increasing need for hepatoprotective agents which would facilitate regeneration by proliferation of parenchymal cells and arrest the growth of fibrous tissue. Evidences suggested that liver injury could be induced by exposure to various pollutants, toxicants, hazardous chemicals and a number of drugs. Carbon tetrachloride, paracetamol, D-galactosamine, rifampicin and thioacetamide are some of the agents that can cause oxidative liver damage. Paracetamol (N-acety1-p-aminophenol) is a widely used analgesic and antipyretic in medical and veterinary practice. At therapeutic doses it is a safe drug. But at toxic levels it leads to liver injury due to oxidative stress mediated by the metabolites of paracetamol. Acerola fruit has antioxidant, anti-diabetic, anti-carcinogenic, immuno protective and anti-aging properties. Even though the chemical composition of the fruit revealed that it can ameliorate oxidative stress, animal studies on the effect of Acerola berry on oxidative damage is very scanty. Hence, study on the *in vivo* effects of this berry is quite promising.

Considering the increasing incidence of hepatic oxidative damage and its impact on animal health and production as well as to identify naturally occurring antioxidants, the present work was undertaken to evaluate antioxidative, hepatoprotective and hepatocurative effect of *Malphigia glabra* on paracetamol induced hepatoxicity in rats. The results of the study will indicate whether it is beneficial to recommend this berry extract in hepatotoxic challenges.

Review of Literature

2. REVIEW OF LITERATURE

Body cells and tissues are continuously threatened by damage caused by free radicals even during normal oxidative processes or by exogenous damage. Free radicals are chemicals that contain one or more unpaired electrons and therefore can act as oxidizing agents. Oxidative stress is an imbalance between cellular production of free radicals and the ability of the cells to defend against them which brings about cell and tissue damage in the body. The major causes of oxidative damage include environmental factors, ingestion of oxidants, inadequate antioxidant intake and enzymatic disorders. Drug/chemical mediated oxidative hepatic injury is the most common manifestation and accounts for greater than 50% of acute liver failure cases (Gillette, 1995, Lee, 2003). Paracetamol can cause nephrotoxicity, extra hepatic lesions and even death in experimental animals, when administered in overdoses (Ray *et al.,* 1996). Its toxicity also represents one of the leading causes of drug induced liver failure by depleting the antioxidant resources (Litovitz *et al.,* 2002).

Antioxidants are substances that intercept free radicals by donating an electron and protect cells from oxidative damage. Some antioxidants are produced in body cells which include enzymes and small molecules like glutathione, uric acid coenzyme Q-10 and lipoic acid. Other essential antioxidants such as vitamin C, E, polyphenols, carotenoids and selenium must be obtained from food. Main sources of natural antioxidants are fruits, vegetables and beverages. Berry fruits such as Acerola berry, Chilean blackberry, Acai berry, Wild blueberry, Bilberry, Cranberry, Elderberry, Strawberry and Goose berry are rich sources of natural antioxidants. The fruits of *Malphigia glabra* (Acerola) are widely available in Kerala, have high vitamin C content, phenolic compounds, carotenoids and anthocyanins all of which contribute to its antioxidant property.

2.1 EFFECTS OF OXIDATIVE STRESS

The direct and indirect effects of reactive oxygen species (ROS) fall into three categories. These include damage of DNA and RNA, lipid peroxidation and protein damage. It has long been thought that the toxic effect of ionizing radiation on cell is mainly due to genomic derangement. As DNA resides in a hydrophilic compartment, ionic radiations can generate hydroxyl radical from nearby water, altering sugar residues and bases that may induce either single or double strand breaks. Biological membranes are highly prone to attack by free radicals as the membrane phospholipids contains a significant proportion of esterified polyunsaturated fattyacids, which are vulnerable to oxidative reactions (Lippman, 1989). The oxygen induced damage is mediated by lipid peroxidation. Products of lipid peroxidation are deleterious to many membrane associated proteins, especially those proteins with exposed tryptophan or cysteine residues. Sulfhydryl groups of these aminoacids react with aldehydes and malondialdehyde (MDA) (end products of lipid peroxidation), causing the development of both intra and intermolecular crosslinks. Lipid peroxidation can also reduce membrane fluidity, thus altering the function of intrinsic proteins. Proteins can also be damaged by direct attack of hydroxyl radical.

2.3 PARACETAMOL TOXICITY

2.3.1 Mechanism of Action

In the liver, paracetamol is converted by hepatic cytochrome P_{450} enzymes to a toxic reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI). This toxic metabolite conjugates rapidly with glutathione (GSH) forming mercapturic acid which subsequently is excreted in the urine (Dahlin *et al.,* 1984, Nelson *et al*., 1990, Lee *et al.,* 2001 and Jodynis-Liebert *et al.,* 2005). However, when paracetamol was overdosed the detoxifying capacity of the liver gets saturated while GSH reserves are depleted and NAPQI accumulate. Exhaustion of GSH

resulted in covalent binding of NAPQI to critical cellular proteins especially mitochondrial proteins (as paracetamol - cysteine adducts) and thus in paracetamol toxicity mitochondria are the primary targets. Ultimately their cellular functions get disrupted by reduced respiration and increased superoxide production and the viability of hepatocytes decreases, leading to cellular necrosis (Cohen and Khairallah, 1997, Kon *et al.*, 2004). Non parenchymal cells such as kupffer cells, natural killer cells and neutrophils were also involved in the generation of ROS during paracetamol induced liver damage (Gardner *et al.*, 2003, Liu *et al.*, 2004). It is observed that ROS such as nitric oxide and superoxide anion are the important determinants for hepatocyte death (Hinson *et al.*, 2004).

2.3.2 Effect of paracetamol toxicity on Body Weight and Haematology

Effect of paracetamol at toxic dose (750 mg/kg b.w.) on energy metabolism of rat liver mitochondria was examined by Katyare and Satav (1989) and reported 21 % decrease in the yield of mitochondrial proteins and a significant reduction in body weight. According to Kumar *et al.* (2001), when hepatotoxicity was induced with paracetamol (750 mg/kg b.w., p.o.) in Wistar rats, the total leukocyte count in the whole blood increased and the body weight decreased significantly.

Bhaumik and Sharma (2002), induced hepatopathy in rabbits by single intravenous injection of paracetamol @ 400 mg/kg b.w. and observed significant decrease in haemoglobin (Hb) concentration, packed cell volume (PCV), and total erythrocyte count (TEC). A considerable increase in total leukocyte count (TLC) and neutrophil count were also noticed. Mathew (2005) observed that when paracetamol was administered to rats @ 3 g/kg b.w., p.o. for three consecutive days produced a significant weight loss. But there was no significant change in haematological parameters. Midhun (2008) also observed a remarkable weight

loss in paracetamol administered rats without any significant changes in TEC, TLC and Hb concentration in whole blood.

2.3.3 Effect of paracetamol toxicity on Enzyme Parameters

According to Dixon *et al.* (1975) toxic dose of paracetamol (4 g/kg b.w., p.o.) in rats produced elevated activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) after 24 hrs of administration. They opined that this was a reliable indication of necrosis of liver. Morais *et al.* (1991) pointed out that paracetamol (750 mg/kg b.w., intraperitonial) in rats produced hepatotoxicity, indicated by significantly high plasma ALT activity. Szymanska *et al*. (1991) administered single dose of acetaminophen (300 mg/kg b.w., intraperitonial) in mice and noticed increased activities of serum ALT and AST suggesting liver damage.

Kaushal *et al.* (1999) evaluated the effects of paracetamol in rats on microsomal function and found that paracetamol (650 mg/kg b.w., intraperitonial) produced six fold increase in serum AST and eight fold increase in serum ALT activities. Bhanwra *et al.* (2000), Asha (2001), Olaleye *et al.* (2006) and Ojo *et al.* (2006), observed a marked elevation of serum ALT, AST and alkaline phosphatase (ALP) activities in adult rats, within 24 to 48 hrs after administration of paracetamol p.o. @ 2 g/kg b.w.

Udupa *et al*. (2000) reported that chronic administration of paracetamol in rats @ 500 and 1000 mg/kg b.w., p.o. for four weeks produced dose dependent increase in ALT and AST activities, indicating the hepatocellular damage. Moore *et al.* (2001) also observed that paracetamol (5 mmol/kg b.w., intraperitonial) in rats caused increase in plasma ALT and AST activities signifying liver damage.

Hepatopathy in rabbits induced by single intravenous injection of paracetamol @ 400 mg/kg b.w., significantly increased serum ALT and AST

activities due to necrosis or altered membrane permeability in liver (Bhaumik and Sharma, 2002). In rats, Chattopadhyay and Bandyopadhyay (2005) observed significant reduction in antioxidant enzymes such as glutathione peroxidase (GPX), glutathione-s-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) representing hepatic damage, on administration with paracetamol p.o. @ 2 g/kg b.w.

According to Mathew, (2005) and Kumar *et al.* (2006), oral paracetamol intoxication in rats @ 3 g/kg b.w. for three days significantly elevated the activities of serum ALT, AST and ALP within 24 hrs, indicating acute hepatotoxicity. Gupta and Misra, (2006) observed that paracetamol @ 200mg/kg b.w. resulted in elevated serum ALT, AST and ALP activities which denoted damage to the hepatic cells. Roy *et al.* (2006) revealed marked increase in serum ALT, AST and ALP activities, on administration of paracetamol @ 1 g/kg b.w. in three divided doses, which was indicative of hepatic damage.

Dash *et al*. (2007) reported that paracetamol @ 750 mg/kg b.w. caused significantly elevated activities of liver specific serum markers like ALT, AST and ALP and decreased the activities of SOD and CAT. Fakurazi *et al.* (2008) administered paracetamol (3 g/kg b.w., p.o.) in 40 % sucrose syrup. They stated that toxicity in rats was initiated by oxidative stress indicated by significant elevation in the activities of serum biochemical markers such as ALT, AST and ALP. Murugesan *et al.* (2008) found that the plasma levels of ALT, AST, ALP, and lipid peroxides were significantly increased in rats by administration of a single dose of carbon tetrachloride (CCl_4) (2 ml/kg b.w., intraperitonial) signifying oxidative hepatic damage.

2.3.4 Effect of paracetamol toxicity on Biochemical Parameters

2.3.4.1 Serum Protein Profile and Albumin:Globulin Ratio

Bhaumik and Sharma (2002), observed a significant decrease in total serum proteins, albumin and albumin:globulin (A:G) ratio, in rabbits with induced hepatotoxicity by single intravenous injection of paracetamol @ 400 mg/kg b.w.. According to Mathew (2005), administration of paracetamol in rats, @ 3 g/kg b.w., p.o. for three consecutive days, resulted in reduced serum total protein and albumin level.

Gupta and Misra, (2006) reported a reduction in total serum protein and liver glycogen which was associated with the decrease in the number of hepatocytes and decreased capacity to synthesize proteins and glycogen and consequently decreased liver weight. Acetaminophen challenge (300 mg/kg b.w., intraperitonial) for seven days produced significant decrease in level of serum total protein (Iwalokun *et al*., 2006). Oral paracetamol intoxication in rats @ 3 g/kg b.w., significantly diminished serum total protein, indicating acute liver failure (Kumar *et al.,* 2006). Dash *et al.* (2007) reported a significant decrease in level of serum total protein in rats administered with paracetamol p.o. @ 750 mg/kg b.w.

2.3.4.2 Serum Total Cholesterol

Bhaumik and Sharma (2002), observed a substantial increase in the levels of serum cholesterol and triglycerides in rabbits where hepatotoxicity was induced by single intravenous injection of paracetamol @ 400 mg/kg b.w. Olaleye *et al.* (2006) observed that paracetamol (2 g/kg b.w., p.o.) produced oxidative liver damage which was indicated by elevated serum cholesterol levels. Ojo *et al.* (2006) also stated that paracetamol induced liver damage in rats produced significant increase in serum cholesterol concentration.

2.3.4.3 Blood Urea Nitrogen (BUN)

Male Fischer rats administered with paracetamol (600 mg/kg b.w., intraperitonial) showed elevated BUN level after 12 hrs of intoxication (Beierschmitt *et al.*, 1986). Morais *et al.* (1991) reported that paracetamol intoxication (750 mg/kg b.w., intraperitonial) in rats produced nephrotoxicity and was assessed by the high concentrations of plasma BUN level.

According to El-Ridi *et al.* (2000) oral administration of paracetamol @ 1 g/kg b.w. in rats induced high mortality rate and hepatorenal toxicity and was indicated by significantly higher levels of BUN and lower levels of liver glutathione. Kumar *et al.* (2001) also reported that when hepatotoxicity was induced in Wistar rats by administration of paracetamol (750 mg/kg b.w. p.o.) there was marked elevation in BUN levels. Isik *et al.* (2005) observed elevated BUN and creatinine levels in paracetamol (1 g/kg b.w., p.o.) treated rats.

2.3.4.4 Serum Total, Direct and Indirect Bilirubin

Bhaumik and Sharma (2002), observed a significant increase in the level of serum bilirubin in rabbits with induced hepatotoxicity by single intravenous injection of paracetamol @ 400 mg/kg b.w. Gupta and Misra, (2006) reported that in rats, administered with paracetamol @ 200mg/kg b.w. produced high level of serum bilirubin which denoted damage to the hepatic cells. Iwalokun *et al*. (2006) observed that acetaminophen challenge (300 mg/kg b.w., intraperitonial) for seven days produced significant increase in level of bilirubin in serum. Oral paracetamol intoxication in rats @ 3 g/kg b.w. significantly elevated the levels of serum total bilirubin indicating acute liver failure (Kumar *et al.*, 2006).

Olaleye *et al.* (2006) observed that in rats, paracetamol (2 g/kg b.w., p.o.) produced oxidative liver damage indicated by elevated serum total and direct bilirubin level. Studies conducted by Roy *et al.* (2006) revealed that

administration of paracetamol in rats @ 1 g/kg b.w. in three divided doses produced marked increase in serum bilirubin level signifying hepatic damage. Dash *et al*. (2007) reported that administration of paracetamol @ 750 mg/kg b.w. in rats resulted in a significant elevation in levels of serum bilirubin.

2.3.4.5 Reduced Glutathione and Lipid Peroxides

Rikans and Kasanka (1984) suggested that GSH plays a major role in determining detoxifying capacity of the organism. A significant decrease in the intracellular GSH content may lead to oxidative stress (Moore *et al.*, 1985, Corcoran and Wong, 1986). In the case of paracetamol toxicity, lipid peroxidation resulting from oxidative stress was responsible for the initiaition and progress of liver damage (Albano *et al.*, 1985). Reduced GSH is a reducing factor in the metabolism of many peroxides and this reduction is catalyzed by GPX (Halliwell and Gutteridge, 1989) and thus GSH plays an important role in maintaining hepatocyte integrity (Comporti *et al.*, 1991, Amimoto *et al.*, 1995).

Flanagan and Meredith (1991) suggested that the hepatorenal toxicity caused by acetaminophen mediated by a reactive metabolite is normally detoxified by reduced glutathione Szymanska *et al.* (1991) administered single dose of acetaminophen (300 mg/kg b.w., intraperitonial) in mice and reported significantly increased level of lipid peroxides and decreased GSH content in liver. Muriel *et al.* (1993) reported a significantly decreased GSH content and increased lipid peroxidation in Wistar male rats suggesting liver damage, after paracetamol intoxication. Also Yonamine *et al*. (1996) observed that paracetamol *in vivo* caused oxidative stress by reduction in liver GSH content at 1 hr after administration which reached its minimum level at 3 hrs.

According to Kuralay *et al.* (1998) and Yanpallewar *et al.* (2002), GSH is a powerful scavenger of free radicals. However, during this process the GSH will get oxidized resulting in further depletion of GSH stores. Therefore, GSH is

important to maintain cellular macromolecules in functional states and it serves as a key determinant of the extent of paracetamol induced hepatic damage. Plewka *et al*. (2000) evaluated the effect of paracetamol (1 g/kg b.w.) on the liver GSH content in rats and reported a significant decrease to less than 55 % of the normal control GSH content at eight hrs of intoxication.

Ahmed and Khater (2001) suggested that preservation of GSH from being depleted provides direct protection against paracetamol induced hepatotoxicity. According to Mathew (2005), administration of paracetamol p.o. in rats, @ 3 g/kg b.w. for three consecutive days, resulted in elevated levels of lipid peroxides. Singh *et al.* (2005) reported a significant increase in the formation of MDA, a byproduct of lipid peroxidation and considered it as an indication of liver toxicity.

Acetaminophen challenge (300 mg/kg b.w., intraperitonial) for seven days produced significant increase in levels of thiobarbituric acid reactive substances (TBARS) and iron (Iwalokun *et al*., 2006). Dash *et al*. (2007) reported that in rats, paracetamol @ 750 mg/kg b.w. resulted in a significant elevation in levels of serum TBARS and decrease in GSH content. Studies conducted by Fakurazi *et al.* (2008) revealed that a high dose of paracetamol remarkably reduced the level of cellular GSH.

2.3.5 Effect of paracetamol toxicity on Liver and Renal Histology

Changes in the liver of rats sacrificed at various intervals after administration of paracetamol overdose were marked congestion, dilatation of central veins, disruption of surrounding sinusoids and frank necrosis of hepatocytes. These histopathological changes were similar in human and rats (Dixon *et al.*, 1971). Beierschmitt *et al.* (1986) reported paracetamol induced renal lesions in the histopathology of male Fischer rats administered with paracetamol overdose (600 mg/kg b.w., intraperitonial).

El-Ridi *et al.* (2000) pointed out cellular alterations and necrosis of hepatocytes and renal cortical cells in rats administered with paracetamol p.o. @ 1 g/kg b.w. Studies conducted by Bhanwra *et al.* (2000) showed marked histopathological changes such as confluent centrilobular necrosis, cloudy swelling, pyknotic nuclei, cytoplasmic matrix swelling and eosinophilic cytoplasm in liver of rats treated with paracetamol p.o. @ 2 g/kg b.w..

Udupa *et al*. (2000) reported that chronic administration of paracetamol in rats @ 500 and 1000 mg/kg b.w. for four weeks produced hydropic degeneration, swelling and necrosis of the liver. According to Ilavarasan *et al.* (2001), microscopical examination of liver of CCl_4 treated rats showed pathological changes such as cloudy swelling, necrosis of hepatic cells and central lobular fatty degeneration.

Hewawasam *et al*. (2003) observed confluent necrosis with vacoulation and ballooning degeneration in the hepatocytes of paracetamol induced hepatotoxic rats. Mathew (2005) reported that paracetamol @ 3 g/kg b.w. for three consecutive days resulted in centrilobular coagulative necrosis of the liver. Kumar *et al.* (2006) also found that oral paracetamol intoxication in rats @ 3 g/kg b.w. resulted in acute centrilobular necrosis of liver.

Isik *et al.* (2005) observed tubular epithelial degeneration, vacuolization, cell desquamation and presence of cellular debris in the proximal tubules and cortical interstitial congestions in the kidneys of paracetamol (1 g/kg, b.w., p.o.) treated rats. Studies conducted by Roy *et al.* (2006) revealed that paracetamol @ 1 g/kg b.w. in three divided doses produced severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis in liver.

Fakurazi *et al.* (2008) observed hepatocellular damage, prominent microvesiculation and moderate infiltration of monocytes and neutrophils with scattered focal necrosis in rat liver induced hepatotoxicity with paracetamol.

Murugesan *et al.* (2008) reported macro and micro vesicular zonal necrosis in the hepatic tissues by CCl_4 (2 ml/kg b.w., intraperitonial) intoxication in rats. Girish *et al.* (2008) noticed extensive necrosis of hepatocytes, which was more pronounced in the centrizonal region with scattered fatty changes and inflammatory reaction in CCl_4 induced hepatotoxic rats.

2.4 NATURAL ANTIOXIDANTS

Antioxidants are substances that delay the oxidation process by inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Halliwell and Aruoma, 1991). Since, synthetic antioxidants require broad and expensive tests to determine their safety for food applications, there is increasing interest in the use of naturally occurring antioxidants (Frankel *et al.*, 1995).

Adverse conditions within the environment, such as smog and UV radiation, in addition to diets rich in saturated fatty acids and carbohydrates, increase oxidative damage in the body. On constant exposure to oxidants, antioxidants may be necessary to counteract chronic oxidative effects, thereby improving the quality of life (Roberts *et al.*, 2003). *In vivo* studies in rats showed that, polyphenolic compounds in plant materials reduced oxidative stress by scavenging ROS and preventing cell damage (Fukuda *et al.*, 2004).

2.4.1 Composition of *Malphigia glabra* (Acerola) Berries

Acerola fruit and derivatives are potent antioxidant foods and are principally known for their high vitamin C content, carotenoids and bioflavonoids all of which contribute for their antioxidant property. Acerola also has high amounts of thiamine, riboflavin, niacin, proteins, and mineral salts, mainly iron, calcium and phosphorous (Vendramini and Trugo, 2000, Mezquita and Vigoa, 2000). It is a tropical fruit which contains various functional compounds other

than vitamin C, such as polyphenols (Kawagishi *et al.,* 2001). Lima *et al.* (2003) stated that the main components of flavonoids in Acerola fruit are anthocyanins (37.9–597.4 mg/kg) and flavonols (70–185 mg/kg)

Acerola berries had high amount of ascorbic acid (1100-1400 mg/100g), anthocyanin (2.7-7.8 mg/100g) and phenolic content (3300-4400 mg/100g) which mainly contributed to its antioxidant property (Filgueiras *et al.,* 2003). Rani *et al.* (2004) assessed the levels of various enzymatic and non-enzymatic antioxidants in selected berries of Indian sub-continent viz., gooseberry, grapes, orange and tomato. The study revealed that all the extracts of berries possess enzymatic antioxidants specifically SOD, CAT and GPX and non-enzymatic antioxidants such as reduced GSH, vitamin C and vitamin A.

Additional major chemical constituents present in Acerola berries are tartaric, malic and citric acid (Righetto *et al*., 2005). Hanamura *et al.* (2005) identified two anthocyanins (cyanidin-3-rhamnoside and pelargonidin-3-rhamnoside) and one flavonol (quercetrin) from these berries. Lima *et al.* (2005) identified high carotenoid level of 3.20 to 4.06 mg/kg in Acerola fruits. According to Mezadri *et al.* (2006) the composition of Acerola fruit (per kg), is, carbohydrates (35.7–78.0 g), proteins (2.1–8.0 g), lipids (2.3–8.0 g), phosphorus (171 mg), calcium (117 mg), iron (2.4 mg), pyridoxine (87 mg), riboflavin (0.7 mg), thiamine (0.2 mg), water (906–920 g) and dietetic fibre (30 g).

Hanamura *et al.* (2006) isolated three polyphenols from this fruit: cyanidin-3-α-O-rhamnoside (C3R) and pelargonidin-3-α-O-rhamnoside (P3R) as anthocyanins, and quercetin 3-α-O-rhamnoside (quercitrin). Kawaguchi *et al.* (2007) reported that Acerola berries originally came from a West Indian island in the Atlantic Ocean, and has high vitamin C content, abundant fructose and malic acid. It has high nutritional value as a potent antioxidant. Also Gruia *et al.* (2008) determined the presence of antioxidants in aqueous berry extract and found that the extract contained ascorbic acid, polyphenols and anthocyanins.

2.4.2 Effect of Naturally Occurring Antioxidants on Body Weight and Haematology

Kumar *et al.* (2001) reported that hepatotoxicity induced with paracetamol (750 mg/kg b.w., p.o.) in Wistar rats, increased the total leukocyte count in the whole blood and decreased the body weight significantly, which was effectively normalized by administering extract of *Tridax Procumbens*. According to Juan *et al.* (2002), *trans*-resveratrol is an antioxidant present in peanuts, grapes and wine. This when administered to rats did not produce any significant effect on body weight and haematological parameters.

Abuelgasim *et al.* (2008) reported reduction in TEC, haemoglobin concentration and bodyweight of rats treated with CCl_4 and this was significantly improved in rats treated with *Lepidium sativum* administration. They opined that loss of body weight may be due to the anorexic effect produced by CCl_4 due to its hepatotoxicity which was cured by the use of *Lepidium sativum*. Moreover the use of the plant alone in the second experiment caused increase in bodyweight which may be attributed to presence of growth promoting factors in the seed of the plant.

2.4.3 Effect of Naturally Occurring Antioxidants on Enzyme Parameters

Chattopadhyay *et al.* (1992) observed a decreased serum AST and ALT activities in paracetamol overdosed rats fed with *Azadirachta indica* leaf extract which indicated the hepatoprotective activity of the extract. They opined that this hepatoprotective property was due to flavonol-o-glycosides present in *Azadirachta indica* leaf extract. This was confirmed by altered activities of serum enzymes. Later Bhanwra *et al.* (2000) reported highly significant reduction in serum AST and ALT activities in rats treated with the neem leaf extract and paracetamol, compared to paracetamol treated alone.

Aktay *et al.* (2000) reported that *Gentiana olivieri* extracts exhibited 86.39% protection for ALT activity and 69.57% protection for AST activity in serum against CCl_4 induced hepatotoxicity in rats. Asha (2001) studied the hepatoprotective activity of *Marmodica subangulata* and found that administration of tender leaves @ 50 mg/kg b.w. markedly prevented paracetamol induced elevation in the activities of serum enzymes such as ALT, AST and ALP.

The oral administration of flavonoids from *Mangifera indica* and *Emblica officinalis* at a dose of 10 mg/kg b.w. in hypercholesterolemic rats showed significant increase on the activities of oxidative free radical scavenging enzymes such as SOD, CAT, GPX, and glutathione reductase (Anila and Vijayalakshmi, 2003)**.** According to Jalalpure *et al*. (2003), ethanolic and butanolic extract of fruits of *Piper longum* @ 300 mg/kg b.w., p.o. for four days offered hepatoprotection in CCl_4 induced hepatotoxicity by decreasing the elevated ALT and AST activities.

Studies conducted by Gupta *et al*. (2004) revealed that, methanolic extract of the plant *Bauhinia racemosa* @ 50, 100 and 200 mg/kg b.w. produced significant hepatoprotective effect on both paracetamol and CCl_4 toxicity by decreasing the activities of serum marker enzymes like ALP, AST and ALT and by significantly increasing the activities of GSH, SOD, and CAT in a dose dependent manner Hewawasam *et al*. (2004) found that pre-treatment of *Epaltes divaricata* extract @ 0.9 g/kg b.w., p.o. for seven days significantly reduced the elevated serum ALT, AST and ALP activities in CCl_4 induced hepatotoxicity.

Kumar *et al*. (2004) evaluated the effect of ethanolic extract of *Trianthema portulacastrum* against paracetamol induced hepatotoxicity in rats. Treatment of rats with the extract @ 200 mg/kg b.w., p.o. for 10 days significantly reduced the increased activities of serum ALT, AST and ALP. Mangathayaru *et al*. (2005) studied the effect of *Leucas aspera* on CCl_4 induced hepatotoxicity in rats and reported that the pretreatment with the dose of 200 and 400 mg/kg b.w.

significantly reduced the elevated activities of liver enzymes, there by showed hepatoprotective action. They also stated that the flavanoids present in the plant was responsible for the hepatoprotective action.

The methanolic extract of the plant *Berberis tinctoria* at the doses of 150 and 300 mg/kg b.w. produced significant hepatoprotective effect on paracetamol induced hepatic damage in rats by decreasing the activities of serum enzymes like ALT, ALP and AST. It significantly increased the CAT and SOD activities in a dose dependent manner (Murugesh *et al*., 2005). Antony *et al*. (2006) studied the hepatoprotective effect of *Centella asiatica* against CCl_4 induced hepatotoxicity and they found that the alcohol extract of *Centella asiatica* , p.o. in two doses 20 and 40 mg/kg b.w. significantly reduced the elevated serum ALT, AST and ALP activities and increased the activities of antioxidant enzymes like SOD, CAT and GPX.

Olalaye *et al.* (2006) conducted the phytochemical screening of *Alchornea cordifolia* leaf extract and revealed the presence of flavanoids. When the extract was administered to paracetamol induced hepatotoxic adult Wistar albino rats, it significantly reduced the serum AST, ALT and ALP activities. Bose *et al*. (2007) evaluated the antioxidant and hepatoprotective effects of *Eupatorium ayapana* in CCl_4 induced hepatotoxicity. It was reported that the methanol extract of the plant @ 200 and 300 mg/kg b.w. produced significant hepatoprotective effect by decreasing the activities of serum enzymes. It also significantly increased the activities of SOD and CAT in a dose dependent manner.

According to Moresco *et al.* (2007) administration of aqueous extract of *Syzygium cumini* to rats @ 0.9 g/kg b.w., p.o. for seven days offered good protection against CCl_4 induced hepatotoxicity, indicated by significant reduction of the increased serum AST and ALT activities. They suggested that free radical scavenging effect of *Syzygium cumini* extract was due to the presence of flavonoids which was responsible for its hepatoprotective effect. Girish *et al.*

(2008) also observed that administration of polyherbal formulation protected the rats from CCl_4 induced hepatic damage, which was evidenced by significantly reduced activities of serum ALT, AST and ALP.

2.4.4 Effect of Naturally Occurring Antioxidants on Biochemical Parameters

2.4.4.1 Serum Protein Profile and Albumin:Globulin Ratio

Ginkgo biloba at the dose of 50 mg/kg b.w., intraperitonial, for seven days increased the serum total protein and albumin levels in treated rats against CCl_4 induced toxicity and confirmed that *Ginkgo biloba* protected the liver from CCl_4 induced liver damage (Shenoy *et al.*, 2001). According to the studies conducted by Gupta *et al*. (2004) the methanol extract of the plant *Bauhinia racemosa* @ 50, 100 and 200 mg/kg b.w. produced significant hepatoprotective effect on both paracetamol and CCl_4 toxicity by significantly increasing the level of serum total protein in a dose dependent manner

Kumar *et al*. (2004) evaluated the effect of ethanolic extract of *Trianthema portulacastrum* against paracetamol induced hepatotoxicity in rats. Treatment of rats @ 200 mg/kg b.w., p.o. for 10 days significantly increased the serum total protein level. Ethanolic extract of the pulp of *Eugenia jambolana* @ 200 mg/kg b.w., p.o. for 10 days showed significant hepatoprotective activity in rats with paracetamol induced hepatotoxicity, evidenced by increased level of serum total protein and enzyme activities (Gayatri and Das, 2006).

Bose *et al*. (2007) evaluated the antioxidant and hepatoprotective effects of *Eupatorium ayapana* in CCl_4 induced hepatotoxicity. It was reported that the methanol extract of the plant @ 200 and 300 mg/kg b.w. produced significant hepatoprotective effect by significantly increasing the level of total protein in a dose dependent manner.

2.4.4.2 Serum Total Cholesterol

Gulati *et al.* (1991) reported that ethanolic extract of roots of *Boerhaavia diffusa* @ 100 mg/100g b.w. for 21 days offered significant hepatoprotection in liquor hepatotoxicity and this was confirmed by the normalized concentrations of triglycerides and cholesterol in extract treated rats. Ravi *et al*. (2005) reported the effect of oral administration of ethanolic extract of *Eugenia jambolana* seed kernel (100 mg/kg b.w.) on the levels of cholesterol, phospholipids, triglycerides and free fatty acids in the plasma, liver and kidney tissues of diabetic rats. It has hypolipidemic effect, which was due to the action of flavonoids, saponins, glycosides and triterpenoids present in the extract.

Ojo *et al.* (2006) showed inhibitive effects of green tea and lemon grass extracts on paracetamol induced liver damage in rats. They stated that pre-treatment of rats with these extracts prevented the alteration in serum cholesterol concentration. They opined that this was mainly due to the antioxidant activity of the extracts to scavenge the free radicals and thus resulting in hepatoprotection. According to Olalaye *et al.* (2006) *Alchornea cordifolia* leaf extract which contains flavanoids, when administered to paracetamol induced hepatotoxic adult Wistar albino rats, significantly reduced the serum cholesterol concentration.

2.4.4.3 Blood Urea Nitrogen (BUN)

Khan *et al.* **(**2001) revealed that prophylactic treatment of rats with *Tephrosia purpurea* at doses of 5 mg/kg b.w. and 10 mg/kg b.w. prevented N-diethylnitrosamine-initiated and potassium bromate- promoted renal oxidative stress and toxicity. This was evinced by the significant reduction in the raised BUN level in a dose-dependent manner.

According to Satyanarayana *et al.* (2001) toxic doses of cyclosporine (20 mg/kg b.w., subcutaneous) for 21 days produced oxidative stress and

nephrotoxicity in rats, which was assessed by elevated BUN level. They opined that administration of quercetin, a bioflavonoid (2 mg/kg b.w.) markedly reduced the elevated BUN level.

Singh *et al.* (2005) and Eybl *et al.* (2008) conducted studies to investigate the effect of quercetin, with antioxidant potential on ferric nitrilotriacetate induced nephrotoxicity in rats. Pretreatment of animals with quercetin (2 mg/kg b.w., intraperitonial) markedly attenuated the BUN level. The result clearly suggested a protective effect of quercetin on nephrotoxicity in rats.

2.4.4.5 Serum Total, Direct and Indirect Bilirubin

Jayasekhar *et al*. (1997) stated that ethyl acetate extract of *Acacia catechu* @ 250 mg/g offered significant hepatoprotection by lowering the increased level of serum bilirubin in CCl_4 treated rats. According to the studies conducted by Gupta *et al*. (2004) the methanolic extract of the plant *Bauhinia racemosa* @ 50, 100 and 200 mg/kg b.w. produced significant hepatoprotective effect on both paracetamol and CCl_4 toxicity by decreasing the serum bilirubin concentration.

Kumar *et al*. (2004) evaluated the effect of ethanolic extract of *Trianthema portulacastrum* against paracetamol induced hepatotoxicity in rats. Treatment with the extract @ 200 mg/kg b.w., p.o. for 10 days significantly reduced the increased level of serum bilirubin. The methanolic extract of the plant *Berberis tinctoria* at the doses of 150 and 300 mg/kg b.w. produced significant hepatoprotective effect on paracetamol induced hepatic damage in rats indicated by decreased serum bilirubin concentration (Murugesh *et al*., 2005). Mankani *et al.* (2005) reported the hepatoprotective activity of ethanolic extract of *Pterocarpus marsupium* @ 25 mg/kg b.w., p.o. in rats for 14 days. *Pterocarpus marsupium* significantly reduced the toxic effect of CCl_4 which was evidenced by restoration of the level of serum bilirubin and enzyme activities to normal.

Alchornea cordifolia leaf extract which contains flavanoids, when administered to paracetamol induced hepatotoxic adult Wistar albino rats, significantly reduced the serum total bilirubin level (Olalaye *et al.*, 2006). Bose *et al*. (2007) evaluated the antioxidant and hepatoprotective effects of *Eupatorium ayapana* in CCl_4 induced hepatotoxicity. It was reported that the methanol extract of the plant @ 200 and 300 mg/kg b.w. produced significant hepatoprotective effect by decreasing the serum bilirubin concentration in a dose dependent manner.

2.4.4.6 Reduced Glutathione and Lipid Peroxides

The antioxidant activity of compounds of plant origin is proportional to their phenolic content (Rice-Evans *et al.*, 1997). Aktay *et al.* (2000) stated that *Gentiana olivieri* extracts exhibited 70.45% protection for plasma lipid peroxide levels against CCl_4 induced hepatotoxicity in rats. El-Ridi *et al.* (2000) investigated the effects of vitamin C against paracetamol induced hepatorenal toxicity in male rats. The administration of vitamin C after paracetamol intoxication decreased the hepatorenal toxicity by normalizing the liver GSH content.

The oral administration of flavonoids from *Mangifera indica* and *Emblica officinalis* at a dose of 10 mg/kg b.w. in hypercholesterolemic rats showed significant increase in reduced glutathione and decrease in lipid peroxide content (Anila and Vijayalakshmi, 2003)**.** According to the studies conducted by Gupta *et al*. (2004) the methanol extract of the plant *Bauhinia racemosa* @ 50, 100 and 200 mg/kg b.w. produced significant hepatoprotective effect on both paracetamol and CCl_4 induced hepatotoxicity by decreasing serum lipid peroxidation. It significantly increased the GSH content in a dose dependent manner. Hewawasam *et al.* (2004) found that pre-treatment with *Epaltes divaricata* extract @ 0.9 g/kg b.w., p.o. for seven days significantly increased the liver reduced glutathione content in CCl_4 induced hepatotoxicity.

The methanolic extract of the plant *Berberis tinctoria* at the doses of 150 and 300 mg/kg b.w. produced significant hepatoprotective effect on paracetamol induced hepatic damage in rats by decreasing serum lipid peroxidation. It significantly increased the GSH content in a dose dependent manner (Murugesh *et al*., 2005). Ojo *et al.* (2006) showed inhibitive effects of green tea and lemon grass extracts on paracetamol induced lipid peroxidation in rats. They opined that this inhibitive effect was mainly due to the antioxidant activity of the extracts to scavenge the free radicals and thus resulting in hepatoprotection.

Alchornea cordifolia leaf extract which contains flavanoids, when administered to paracetamol induced hepatotoxic adult Wistar albino rats, markedly increased the GSH content (Olalaye *et al.*, 2006). Bose *et al*. (2007) evaluated the antioxidant and hepatoprotective effects of *Eupatorium ayapana* in CCl_4 induced hepatotoxicity. It was reported that the methanol extract of the plant @ 200 and 300 mg/kg b.w. produced significant hepatoprotective effect by decreasing the activity of lipid peroxides. It also significantly increased the GSH content in a dose dependent manner.

Moresco *et al.* (2007) reported that aqueous extract of *Syzygium cumini* @ 0.9 g/kg b.w., p.o. in rats for seven days offered good protection against CCl_4 induced hepatotoxicity by scavenging of free radicals and thus inhibited the lipid peroxidation resulting in hepatoprotection. Gruia *et al.* (2008) monitored the levels of lipid peroxides in various tissues isolated from male Wistar albino rats weighing 200-220 g, in two experimental models. There was no significant difference concerning the serum lipid peroxidation index (LPI) between the berry extract treated lot and the normal control lot. But the level of hepatic LPI was almost twice as high in the case of berry extract fed animals.

2.4.5 Effect of Naturally Occurring Antioxidants on Liver and Renal Histology

Azadirachta indica leaf extract, provided hepatoprotection in paracetamol overdosed rats. This was confirmed by the reduction in the necrotic areas of liver histology (Chattopadhyay *et al.*, 1992). El-Ridi *et al.* (2000) suggested that administration of vitamin C after paracetamol intoxication decreased the hepatorenal toxicity. It protected hepatic and renal cells except for slight dilatation of rough endoplasmic reticulum and glycogen depletion in some hepatocytes. Vitamin C also prevented against the lethal effect of paracetamol overdose, although it incompletely protected against hepatorenal toxicity.

Singh *et al.* (2005) conducted a study to investigate the effect of quercetin, a bioflavonoid with antioxidant potential on ferric nitrilotriacetate induced nephrotoxicity in rats. Pretreatment of animals with quercetin (2 mg/kg b.w., intraperitonial) markedly reduced deterioration of renal architecture and this clearly suggested a protective effect of quercetin. Mir *et al*. (2007) reported that the alcoholic extract of *Emblica officinalis* @ 100 mg/kg b.w., p.o. for eight weeks reversed the CCl_4, paracetamol and thioacetamide induced abnormal histopathology on liver cells by its ability to accelerate the regenerative activity.

Girish *et al.* (2008) noticed that administration of polyherbal formulation in rats protected the liver from CCl_4 induced damage such as extensive necrosis of hepatocytes, which was more pronounced in the centrizonal region with scattered fatty changes and inflammatory reaction. This was evidenced by restoration of a near normal architecture of the liver parenchyma. Fakurazi *et al.* (2008) observed that, administration of *Moringa oleifera* leaf extract significantly reduced the hepatocellular damage in rat liver induced hepatotoxic with paracetamol. According to Murugesan *et al.* (2008), feeding rats with kombucha tea, a good source of antioxidants, after induction of oxidative hepatic damage with CCl_4,

exhibited a pronounced reduction in the macro and micro vesicular zonal necrosis of liver.

2.4.6 Other Effects of Naturally Occurring Antioxidants

Some tropical and subtropical foods have strong suppressing effect on carcinogenesis and liver injury and hence they are attractive sources of functional foods beneficial to health. (Kawagishi *et al.,* 2001). In terms of function, Nagamine *et al*. (2002) reported that Acerola extract had an inhibitory effect on nitric oxide production in mouse macrophage-like cells and an antitumor effect against lung cancer.

The polyphenolic compounds in edible plants are regarded as natural antioxidants and their antioxidant activities are important for physical condition (Sabu *et al.,* 2002). In mice, anthocyanin of edible berries possessed broad spectrum of anti-angiogenic, antioxidant and anti-carcinogenic properties (Bagchi *et al*., 2004). Hanamura *et al.* (2005) reported that the polyphenolic compounds from tropical plants have both antioxidant and hypoglycemic activities. According to Hanamura *et al.* (2006) Acerola polyphenols (APs) were found to have radical scavenging activities and inhibitory effects on both α-glucosidase and advanced glycation endproduct (AGE) production.

Cespedes *et al.* (2007) reported that, extracts from the fruits of Chilean blackberry possessed antioxidant and cardioprotective activities in rats, which were strongly correlated with its polyphenol content. Studies conducted by Dash *et al*. (2007) revealed that silymarin, a flavolignan from *Silybum marianum,* had hepatoprotective effects in acute viral hepatitis and intoxication with paracetamol, carbon tetrachloride and ethanol. Its mechanism of action included inhibition of hepatotoxin binding to receptor sites on hepatocyte membrane, reduction of glutathione oxidation to enhance its content in the liver and antioxidant activity.

Mai *et al.* (2007) after study on 28 materials of plant extracts reported that the plant extracts rich in polyphenol contents had strong antioxidant activity. The role of polyphenolic compounds in the antioxidant activities indicated that the antioxidant capacity of vegetables depends on the amount of flavonoids that each vegetable possesses. The plant materials with high polyphenol contents had both strong α-glucosidase inhibitory activity and strong antioxidant activity. Kawaguchi *et al.,* (2007) isolated the novel flavonoid, aceronidin, from green mature Acerola puree. Aceronidin showed antioxidative activity, as well as inhibitory activity against sugar catabolic enzymes such as α-glucosidase and α-amylase which lead to retardation of carbohydrate digestion.

Anthocyanins are a group of naturally occurring pigments responsible for the red-blue colour of fruits and vegetables. The potential effect of this family of flavonoids reduced the incidence of cancer, cardiovascular diseases, hyperlipidemias and other chronic diseases through the intake of anthocyanin rich foods (Pascual-Teresa and Sanchez-Ballesta, 2007). According to Hanamura and Aoki, (2008) Acerola polyphenols (APs) were found to have free radical scavenging activities and inhibitory effects on both *α*-glucosidase and AGE production.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

The study was conducted in 42 adult male Wistar rats, weighing 150-200g. The rats were procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. The animals were maintained in well ventilated cages in the Department of Veterinary Physiology under standard managemental conditions for one week, so as to get acclimatized with the new environment. The animals were provided with *ad libitum* feed and water.

3.2 PLANT MATERIALS

The fruit under study was *Malphigia glabra*, commonly called as Acerola berry. Fresh fruits were collected from Thrissur district of Kerala and were authenticated by College of Horticulture, KAU, Vellanikkara (Plate-1 & 2).

3.2.1 Preparation of Aqueous Extract of *Malphigia glabra* (Acerola) Berries

Mature fresh Acerola berries were cleaned and aqueous extract of the fruit pulp was prepared in 1:5 dilution, by diluting 25 ml of finely crushed pulp of Acerola in 125 ml of distilled water. The extract was prepared freshly everyday.

3.2.2 Sugar Syrup

Sugar syrup was prepared by dissolving 40 g of sucrose in 100 ml of water (40 % sugar syrup) and it was used as the vehicle for the administration of drug.

Plate - 1. Plant of *Malphigia glabra* (Acerola)

Plate - 2. Mature ripened fruit of *Malphigia glabra*

3.3 EXPERIMENTAL DESIGN

The experiment was conducted for a period of 21 days. The rats were randomly divided into five groups (G1 to G5) comprising eight animals each in G1, G2 and G5 and nine animals in G3 and G4.

Group 1 (G1) - Control group. The animals were fed with standard rat ration.

Group 2 (G2) - Untreated; Vehicle (sugar) alone. Rats were administered with 40 % sugar syrup p.o. @ 10 ml/kg b.w. on day 1 and 2 and then at 3 days interval for 21 days.

Group 3 (G3) - Untreated (Paracetamol alone). Rats were administered with paracetamol p.o. @ 2 g/kg b.w. on day 1 and 2 and then at 3 days interval upto day 21.

Group 4 (G4) - Hepatocurative group - Paracetamol was administered p.o. @ 2 g/kg b.w. on day 1 and 2 and then at 3 days interval upto day 21. Acerola berry extract was administered throughout the experiment (21 days) p.o. @ 20 ml/kg b.w.

Group 5 (G5) - Hepatoprotective (prophylactic) group - Acerola berry extract was administered p.o. @ 20 ml/kg b.w. for 17 days and paracetamol was administered p.o. @ 2 g/kg b.w. on day 18 and 19.

The blood was collected from all the animals on day zero, four, 10 and 21. From G5 animals, an additional blood collection was done on day 18. Blood as well as separated serum were used for haematological and serum biochemical analysis.

One animal each, from G3 and G4 was euthanized on day four and rest of the rats were sacrificed on day 21. Samples of kidney and liver were collected. Representative samples of these tissues were subjected to histopathological examination. Liver tissues from all the rats were used for biochemical analysis.

3.4 COLLECTION OF BIOLOGICAL SAMPLES

3.4.1 Collection of Blood and Separation of Serum

Animals were subjected to mild ether anaesthesia and blood samples (2 ml/rat) were collected from the retro-orbital plexus using capillary tubes. Samples were collected with and without anticoagulant (Sodium EDTA @ 2 mg/ml of blood). Serum samples were separated and stored at –20ºC for biochemical analysis. Enzyme estimations were done using fresh serum samples on the day of blood collection.

3.4.2 Collection of Liver and Kidney

The animals were euthanized by overdose of ether, dissected and the samples of liver and kidney were collected. They were washed in running tap water to remove the blood clots and representative tissue samples were fixed in 10 % formalin for histopathological examination. Liver tissue samples from all the rats were kept in chilled normal saline (0.9 % sodium chloride) for biochemical analysis.

3.5 PARAMETERS STUDIED

3.5.1 Body Weight

The body weights of the rats were recorded at weekly intervals from day zero to 21 of the experiment.

3.5.2 Haematological Parameters

Total erythrocyte count (TEC), haemoglobin (Hb) concentration, total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated as per the standard technique described by Schalm (1986).

3.5.3 Biochemical Parameters

3.5.3.1 Serum Enzymes

Serum activities of enzymes such as Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) were estimated by the photometric method described by Alan (1988) using Ecoline® kit (M/s. E. Merck India limited, Mumbai).

3.5.3.2 Serum Protein Profile and A:G Ratio

Total serum proteins and serum albumin were estimated by Biuret method (Henry *et al*., 1957) and Doumas method (Doumas *et al*., 1971) respectively using Ecoline® kit (M/s. E. Merck India limited, Mumbai). The serum globulin content was determined by subtracting serum albumin level from total serum protein content and subsequently A:G ratio was calculated.

3.5.3.3 Serum Total Cholesterol

Serum total cholesterol concentration was estimated by Cholesterol Oxidase Peroxidase method suggested by Richmond (1973) using Ecoline® kit (M/s. E. Merck India Limited, Mumbai).

3.5.3.4 Blood Urea Nitrogen (BUN)

The BUN level was estimated by the photometric method described by Alan (1988) using Ecoline® kit (M/s. E. Merck India Limited, Mumbai).

3.5.3.5 Serum Total, Direct and Indirect Bilirubin

The Ecoline® kit from Merck Specialities Pvt. Ltd., was used for the estimation of serum total, direct and indirect bilirubin. Total bilirubin was measured photometrically with the addition of alkali and direct bilirubin was measured without the addition of alkali as described by Alan (1988). Indirect bilirubin was calculated from the difference between total and direct bilirubin.

3.5.4 Antioxidant Profile

3.5.4.1 Chemicals

Trichloroacetic acid (TCA) was procured from Qualigens Fine Chemicals, Glaxo Smith Kline Pharmaceuticals Ltd, Mumbai. Thiobarbituric acid (TBA), 1,1,3,3 Tetra methoxy propane (TMP), disodium hydrogen phosphate, monosodium dihydrogen phosphate and 5,5’ dithiobis- 2- nitrobenzoic acid (DTNB) were purchased from Himedia Laboratories Pvt Ltd, Mumbai.

3.5.4.1.1 Serum and Liver Reduced Glutathione Activity

Levels of reduced glutathione in serum and liver homogenate were determined by the method of Moron *et al.* (1979).

a. Principle

Reduced glutathione was measured by its reaction with DTNB to give a yellow coloured complex with absorption maximum at 412nm.

b. Reagents

1. Phosphate buffer - 0.2 M, pH 8.0

Solution A: Dissolved 3.12 g of $NaH_2PO_4.2H_2O$ in 100 ml distilled water

Solution B: Dissolved 28.39 g of Na_2HPO_4 in 100 ml distilled water

Mixed 5.3 ml of solution A and 94.7 ml of solution B

2. Trichloro acetic acid – 5 % in distilled water

3. Trichloro acetic acid – 25 % in distilled water

4. DTNB - 0.6 mM in distilled water

c. Procedure

1. Preparation of liver homogenate

Liver homogenates were prepared with 0.5 g of wet tissue in 4.5 ml of phosphate buffer. Centrifuged at 5000 rpm and the supernatant was used for the estimation of reduced glutathione.

2. 125 µl of 25 % TCA was added to 500 µl of supernatant of liver homogenate (500 µl of serum for the estimation of serum reduced glutathione activity), for the precipitation of proteins and mixed well.

3. The tubes were then cooled in ice bath for 5 min.

4. The mixture was again diluted with 575 µl of 5 % TCA and centrifuged for 5 min. at 5000 rpm.

5. 300 µl of the supernatant was transferred into another test tube and 700 µl of phosphate buffer was added to it.

6. To the above mixture, 2ml of freshly prepared DTNB was added, mixed well and the yellow colour developed was read at 412 nm.

d. Preparation of standard curve

Standard curve of glutathione was prepared by using concentrations varying from 1-10 µg of glutathione standard which was dissolved in 5 % TCA. The volume of standard solution was made up to 1 ml with 0.2 M phosphate buffer. Added 2 ml of freshly prepared 0.6 mM DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. A graph was plotted between optical density and concentration of the standards. The optical density of the unknown samples was read directly from the calibration curve and expressed as µg/ml for serum and µg/g wet tissue in case of liver.

3.5.4.1.2 Estimation of Lipid Peroxides in Liver

The levels of lipid peroxides in liver were estimated by the method of Fraga (1988).

a. Principle

Thiobarbituric acid (TBA) reacts with malondialdehyde, an end product of fatty acid peroxidation to form a red coloured pigment, which has maximum absorbance at 532nm. 1,1,3,3 tetra methoxy propane was used as standard since it can be converted to malondialdehyde by reacting with TBA.

b. Reagents

1. Trichloro acetic acid (TCA) – 15 % in distilled water
2. Thiobarbituric acid (TBA) - 0.38 % in hot distilled water
3. Hydrochloric acid (HCL) - 0.25 N

4. TCA-TBA-HCl reagent solution - 1, 2 and 3 were mixed freshly in the ratio of 1:1:1

5. Tris – HCl buffer (pH 7.5) - Dissolved 6.85 g Tris in 40 ml distilled water (A) and 1 ml of 12 N HCl was made upto 100 ml (B). Mix solution A and B and adjust the pH to 7.5

6. Standard solution - 1,1,3,3 tetra methoxy propane (4.8 mM)

c. Procedure

1. Freshly excised liver tissue (500 mg) was homogenized with 4.5 ml Tris -HCl buffer (pH 7.5).

2. One ml of tissue homogenate was transferred into a clean test tube and mixed thoroughly with 2 ml of TBA- TCA- HCl reagent.

3. The mixture was placed in boiling water bath for 15 min, cooled and centrifuged at 3200 rpm for 10 min. Finally, the supernatant was taken for measurement.

4. The absorbance of the chromophore was read at 532 nm against the TBA-TCA- HCl reagent blank using spectrophotometer.

d. Preparation of standard curve

Standard curve was prepared using concentrations varying from 0.5 nM to 5 nM of 1,1,3,3 tetra methoxy propane in double distilled water by following the above procedure. A graph was plotted with optical density and concentration of the standards. The level of lipid peroxides were read directly from the standard curve, and expressed as nM of malondialdehyde/g of liver tissue.

3.5.4.1.3 Estimation of Superoxide Dismutase (SOD) in Liver

Superoxide dismutase activity was estimated by the method of Winterbourn *et al.* (1975).

a. Principle

The activity was measured based on the ability of SOD to inhibit the reduction of nitro- blue tetrazolium (NBT) by superoxide.

b. Reagents used

1. Sodium EDTA – NaCN solution - Dissolved 0.0015 g NaCN in 100 ml of 0.1 M EDTA

2. NBT (Nitroblue tetrazolium) - 1.5 mM

3. Phosphate buffer (67 mM, pH 7.8)

Solution A: Dissolved 0.936 g of $NaH2PO_4.2H_2O$ in 100 ml distilled water

Solution B: Dissolved 0.95 g of Na_2HPO_4 in 100 ml distilled water

Mixed 8.5 ml of solution A and 91.5 ml of solution B

4. Riboflavin – 2 mM

c. Procedure

1. 500 mg liver tissue was homogenized with phosphate buffer.

2. 0.1 ml of homogenate was mixed with 0.2 ml of 0.1 M Sodium EDTA, 0.1 ml of 1.5 mmol NBT and phosphate buffer in a total volume of 2.6 ml.

3. Added 0.05 ml of riboflavin and the absorbance of the solution were determined against distilled water as blank at 560 nm.

4. All the tubes were uniformly illuminated for 15 min and the absorbance of the blue colour formed was measured again at 560 nm.

5. Amount of inhibition was calculated after comparing absorbance of sample with that of control.

Values were expressed in U/mg of protein.

3.5.4.1.3.1 Determination of Tissue Protein

Tissue protein was determined according to the method of Lowry *et al.* (1951).

a. Reagents

1. Alkaline Copper sulphate (0.5 % $CuSO_4$ in 1 % sodium potassium tartrate and 2 % sodium carbonate in 0.1 N sodium hydroxide, mixed in the ratio of 1:50)

2. Phosphate buffer (67 mmol, pH 7.8)

3. Folin phenol reagent (1 N)

b. Procedure

1. 500 mg of liver tissue was homogenized in 4.5 ml of phosphate buffer.

2. 0.01 ml of the prepared tissue homogenates were transferred into fresh test tubes and made up to 1ml with distilled water.

3. 5 ml of alkaline copper sulphate was added and kept for 10 min. at room temperature.

4. 0.5 ml of 1 N folin phenol reagent was then added to the mixture.

5. The absorbance was measured after 20 min. at 660 nm against the reagent blank (distilled water treated in similar manner as that of homogenate).

6. Protein content was calculated from the standard graph prepared using different concentrations (0.1 to 0.5 mg/ml) of bovine serum albumin (BSA).

3.5.5 Histopathological Examination

Representative tissue samples from liver and kidney were fixed in 10 % formalin and were processed and embedded in paraffin as described by Sheehan and Hrapshack (1980). Microtome sections were prepared from each tissue and stained with haematoxylin-eosin as per the staining techniques by Bancroft and Cook (1995) to study the histopathological changes.

3.6 STATISTICAL ANALYSIS OF DATA

The results obtained were analysed using Analysis of Co-variance method followed by Duncan's multiple range tests for comparison between groups as described by Snedecor and Cochran (1994).

Results

4. RESULTS

4.1 BODY WEIGHT

The weekly body weight of rats in all the groups (G1, G2, G3, G4 and G5) from day zero to 21 of the experiment is presented in the Table 1 and Fig. 1. There was no significant ($P > 0.05$) difference in the mean body weight on day zero, seven, 14 and 21, between any groups of rats except for a significantly ($P \leq 0.05$) increased body weight in G1 rats (180.00 ± 1.64g), on day 21. Significantly ($P \leq 0.05$) elevated body weight was observed in all the group of rats on day seven, 14 and 21 when compared to the body weight on day zero.

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4.2 HAEMATOLOGICAL PARAMETERS

4.2.1 Total Erythrocyte Count (TEC)

The TEC value recorded at different period of experiment is listed in Table 2 and Fig. 2. On day zero, four and 10 of the experiment, no significant ($P > 0.05$) difference was observed in TEC value between the five groups of rats. On 21st day, a significant ($P \leq 0.05$) reduction in TEC value was noticed in the paracetamol induced hepatotoxic group of rats G3 (6.92 ± 0.17 ×10^6/µl) when compared to G1 (7.586 ± 0.30 ×10^6/µl), G2 (8.024 ± 0.21 ×10^6/µl), G4 (7.795 ± 0.23 ×10^6/µl) and G5 (7.815 ± 0.24 ×10^6/µl) rats. The G3 rats had a significantly ($P \leq 0.05$) reduced TEC on day 21 when compared to day zero. In all other groups, no significant ($P > 0.05$) variation was observed in TEC value on day four, 10 and 21, when compared to day zero.

4.2.2 Haemoglobin (Hb) Concentration

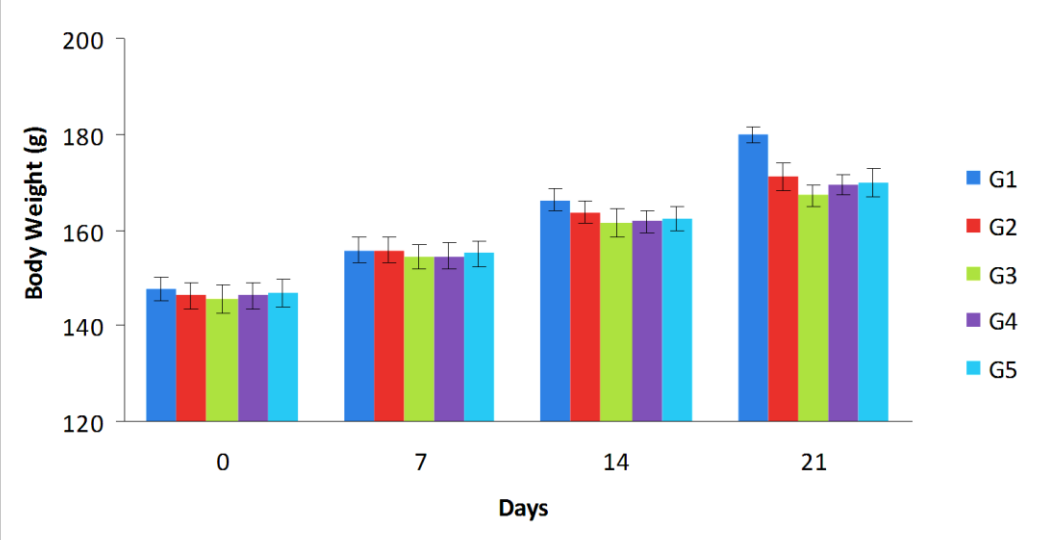
The haemoglobin concentration recorded in different groups of rats, G1, G2, G3, G4 and G5 is shown in Table 3 and Fig. 3. On day zero and four, there

Table 1. Effect of aqueous extract of Acerola berries on body weight of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Body Weight (g)			
	0	7	14	21
G1	147.63 [a] ±2.58	155.63 [a]*±2.74	166.25 [a]*±2.27	180.00 [b]*±1.64
G2	146.25 [a] ±2.63	155.63 [a]*±2.74	163.75 [a]*±2.45	171.25 [a]*±2.95
G3	145.71 [a] ±2.97	154.29 [a]*±2.54	161.43 [a]*±3.03	167.14 [a]*±2.40
G4	146.25 [a] ±2.63	154.38 [a]*±2.74	161.88 [a]*±2.30	169.38 [a]*±2.20
G5	146.88 [a] ±2.98	155.00 [a]*±2.83	162.50 [a]*±2.50	170.00 [a]*±2.89

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, seventh, 14th and 21st.

Fig. 1. Effect of aqueous extract of Acerola berries on body weight of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



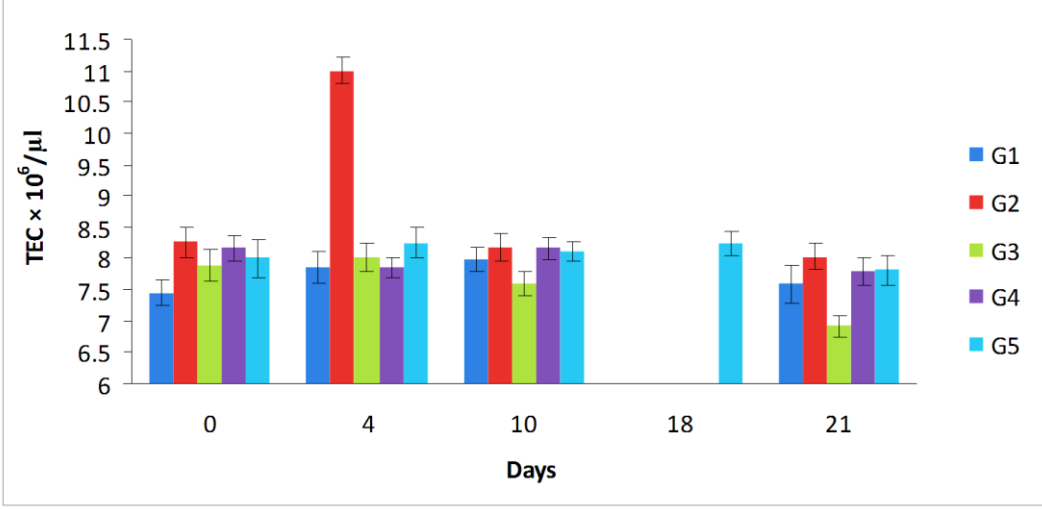
G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Table 2. Effect of aqueous extract of Acerola berries on total erythrocyte count (TEC) of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	TEC × 10^6/µl				
	0	4	10	18	21
G1	7.45 [a] ±0.20	7.85 [a] ±0.26	7.97 [a] ±0.19	-	7.59 [ab] ±0.30
G2	8.27 [a] ±0.24	8.14 [a] ±0.21	8.17 [a] ±0.23	-	8.02 [b] ±0.21
G3	7.88 [a] ±0.26	8.01 [a] ±0.22	7.60 [a] ±0.18	-	6.92[a]*±0.17
G4	8.16 [a] ±0.21	7.85 [a] ±0.17	8.16 [a] ±0.18	-	7.80 [b] ±0.23
G5	8.00 [a] ±0.29	8.24 [a] ±0.24	8.12 [a] ±0.16	8.25±0.19	7.82 [b] ±0.24

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 2. Effect of aqueous extract of Acerola berries on total erythrocyte count (TEC) of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

was no significant (P> 0.05) difference in Hb concentration between different groups of rats. A significantly (P≤ 0.05) reduced Hb concentration was observed on day 10 of the experiment, in paracetamol induced hepatotoxic (G3) rats (11.29 ± 0.28 g %), and the treated group G4 (12.11 ± 0.38 g %), in which the rats were administered both paracetamol and Acerola extract concomitantly. The control rats showed a significantly (P≤ 0.05) higher value (13.52 ± 0.67 g %).

Significantly (P≤ 0.05) reduced Hb concentration was noticed in G3 (10.92 ± 0.32 g %) group of rats when compared to all other groups on 21st day of the experiment. The G2 (12.38 ± 0.21 g %), G4 (12.74 ± 0.36 g %) and G5 (12.01 ± 0.36 g %) rats showed a significantly (P≤ 0.05) reduced Hb value when compared to G1 (13.79 ± 0.54 g %) rats. On day 10 and 21, the G3 rats showed significant (P≤ 0.05) decrease in the Hb concentration when compared to the initial day reading.

4.2.3 Total Leukocyte Count (TLC)

The observed mean TLC value of the control and experimental groups of rats is presented in Table 4 and Fig. 4. Before the start of the experiment, on day zero, no significant (P> 0.05) variation was observed in the TLC value between any groups of rats. On day four also, no significant (P> 0.05) changes were observed in TLC between all the five groups of rats. There was a significant (P≤ 0.05) increase of TLC in G3 (11.51 ± 0.43 ×10^3/µl) rats on the 10th day of the experiment when compared to all other groups rats which showed similar values.

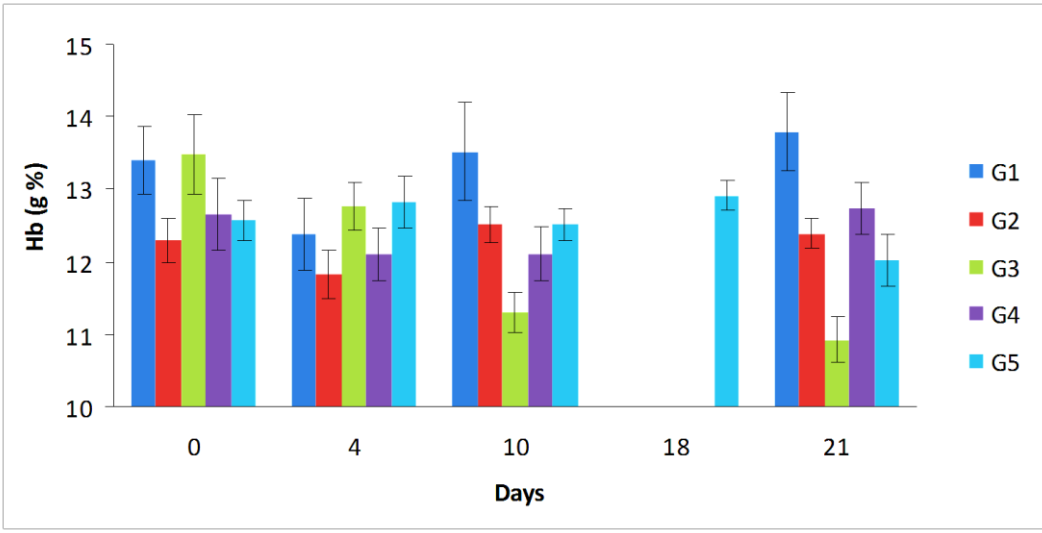
On day 21, rats belonging to G3 (11.29 ± 0.49 ×10^3/µl) and G5 (10.55 ± 0.39 ×10^3/µl) groups showed a significantly (P≤ 0.05) higher TLC, when compared to G1 (8.12 ± 0.36 ×10^3/µl), G2 (8.11 ± 0.22 ×10^3/µl) and G4 (7.91 ± 0.16 ×10^3/µl) rats. The TLC value of G3 group of rats was significantly (P≤ 0.05) high on day 10, when compared to day zero TLC value. The G3 and G5 rats on

Table 3. Effect of aqueous extract of Acerola berries on haemoglobin (Hb) concentration of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Hb concentration (g %)				
	0	4	10	18	21
G1	13.40 ^{a} ±0.46	12.38 ^{a} ±0.49	13.52 ^{b} ±0.67	-	13.79 ^{c} ±0.54
G2	12.29 ^{a} ±0.30	11.82 ^{a} ±0.34	12.52 ^{ab} ±0.25	-	12.38 ^{b} ±0.21
G3	13.48 ^{a} ±0.55	12.77 ^{a} ±0.33	11.29 ^{a}*±0.28	-	10.92 ^{a}*±0.32
G4	12.66 ^{a} ±0.50	12.09 ^{a} ±0.36	12.11 ^{a} ±0.38	-	12.74 ^{bc} ±0.36
G5	12.57 ^{a} ±0.27	12.82 ^{a} ±0.35	12.51 ^{ab} ±0.22	12.91±0.21	12.01 ^{b} ±0.36

Values bearing different superscripts (a, b and c) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st

Fig. 3. Effect of aqueous extract of Acerola berries on haemoglobin (Hb) concentration of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



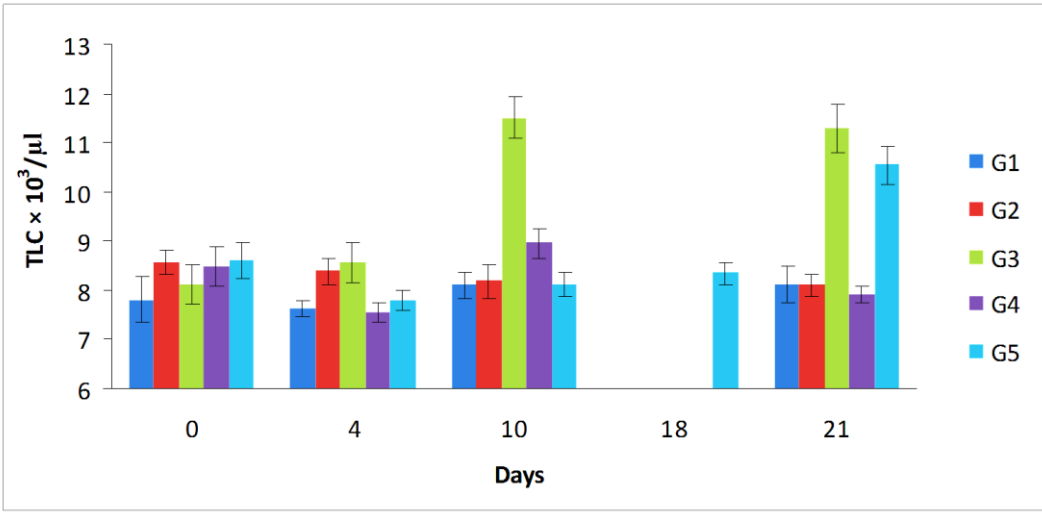
G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Table 4. Effect of aqueous extract of Acerola berries on total leukocyte count (TLC) of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	TLC × 10^3/µl				
	0	4	10	18	21
G1	7.80 [a] ±0.47	7.63 [a] ±0.15	8.10 [a] ±0.27	-	8.12 [a] ±0.36
G2	8.56 [a] ±0.23	8.39 [a] ±0.26	8.18 [a] ±0.36	-	8.11 [a] ±0.22
G3	8.12 [a] ±0.41	8.57 [a] ±0.40	11.51 [b]*±0.43	-	11.29 [b]*±0.49
G4	8.49 [a] ±0.40	7.56 [a] ±0.21	8.96 [a] ±0.31	-	7.91 [a] ±0.16
G5	8.61 [a] ±0.36	7.79 [a] ±0.20	8.11 [a] ±0.25	8.35±0.22	10.55 [b]*±0.39

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 4. Effect of aqueous extract of Acerola berries on total leukocyte count (TLC) of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

day 21, showed a significantly (P≤ 0.05) elevated TLC value when compared to the initial count.

4.2.4 Differential Leukocyte Count (DLC)

The results of DLC obtained are presented in Table 5 and Fig. 5. On the final day (21st day) of experiment, the total neutrophil, lymphocyte and eosinophil count of all the five groups did not differ significantly (P> 0.05) and the basophil count was nil. The monocyte count was found to be significantly (P≤ 0.05) higher in paracetamol administered group, G3 (6.67 ± 1.20%) and the treated group, G5 (6.00 ± 0.53%), in which Acerola extract was administered initially for 17 days and then followed with toxic dose of paracetamol on day 19 and 20.

4.3 BIOCHEMICAL PARAMETERS

4.3.1 Serum Enzymes

4.3.1.1 Alanine Amino Transferase (ALT)

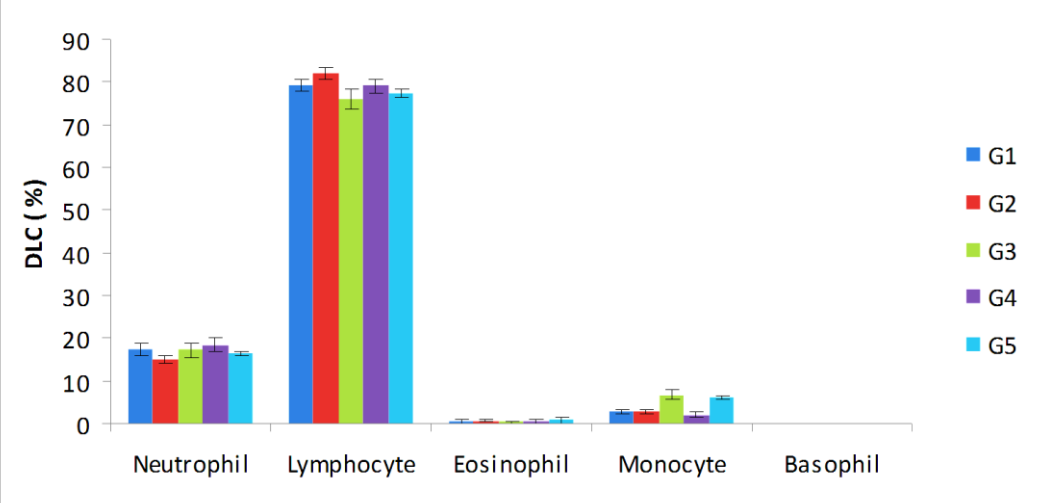
The mean serum ALT activity of control and experimental group of rats are presented in Table 6 and Fig. 6. The initial values of ALT activity recorded on day zero did not show any significant (P> 0.05) variation between the different groups of rats. However, on day four of the experiment (48 hrs after administration of paracetamol) a significant (P≤ 0.05) increase was noticed in the ALT activity of paracetamol induced hepatotoxic (G3) group of rats (177.04 ± 15.21 IU/l) when compared to all other group of rats. The G4 rats that received Acerola extract along with paracetamol also had an ALT activity (68.63 ± 5.15 IU/l) significantly (P≤ 0.05) higher than that of G1 rats (41.28 ± 1.65 IU/l), but the value was similar to that of vehicle administered G2 (56.31 ± 3.82) rats.

Table 5. Effect of aqueous extract of Acerola berries on differential leukocyte count (DLC) of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Groups	DLC %				
	Neutrophil	Lymphocyte	Eosinophil	Monocyte	Basophil
G1	17.33 [a] ±1.36	79.33 [a] ±1.45	0.50 [a] ±0.34	2.83 [a] ±0.48	0
G2	15.00 [a] ±1.03	82.00 [a] ±1.32	0.67 [a] ±0.21	2.67 [a] ±0.49	0
G3	17.17 [a] ±1.62	76.00 [a] ±2.38	0.27 [a] ±0.15	6.67 [b] ±1.20	0
G4	18.50 [a] ±1.82	79.00 [a] ±1.77	0.50 [a] ±0.34	2.00 [a] ±0.63	0
G5	16.50 [a] ±0.57	77.33 [a] ±1.02	0.80 [a] ±0.58	6.00 [b] ±0.53	0

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups.

Fig. 5. Effect of aqueous extract of Acerola berries on differential leukocyte count of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

On day 10 also the G3 rats showed a significantly (P≤ 0.05) high ALT activity (160.65 ± 9.43 IU/l) when compared to other groups. The ALT activity of G4 rats (54.36 ± 11.19 IU/l) was similar to that of G2 (60.90 ± 2.43 IU/l) rats. The G5 rats which received Acerola extract alone, had an ALT activity of 43.50 ± 2.58 IU/l, similar to that of G1 (42.19 ± 3.12 IU/l) rats. On day 21, the G3 rats had an ALT activity (202.77 ± 25.90 IU/l) significantly (P≤ 0.05) higher than that of G1 (44.02 ± 2.01 IU/l), G2 (62.29 ± 4.43 IU/l) and G4 (65.52 ± 4.19 IU/l) rats. The G5 rats fed with paracetamol on day 18 and 19, and Acerola from zero to 17 days also had a significantly (P≤ 0.05) high ALT activity on 21st day (191.97 ± 29.81 IU/l) of the study.

Throughout the experiment, the ALT activity of G3 rats was significantly (P≤ 0.05) high when compared to initial ALT activity. In G5 rats, the activity was significantly (P≤ 0.05) higher on day 21, when compared to all other days of the experiment.

4.3.1.2 Aspartate Amino Transferase (AST)

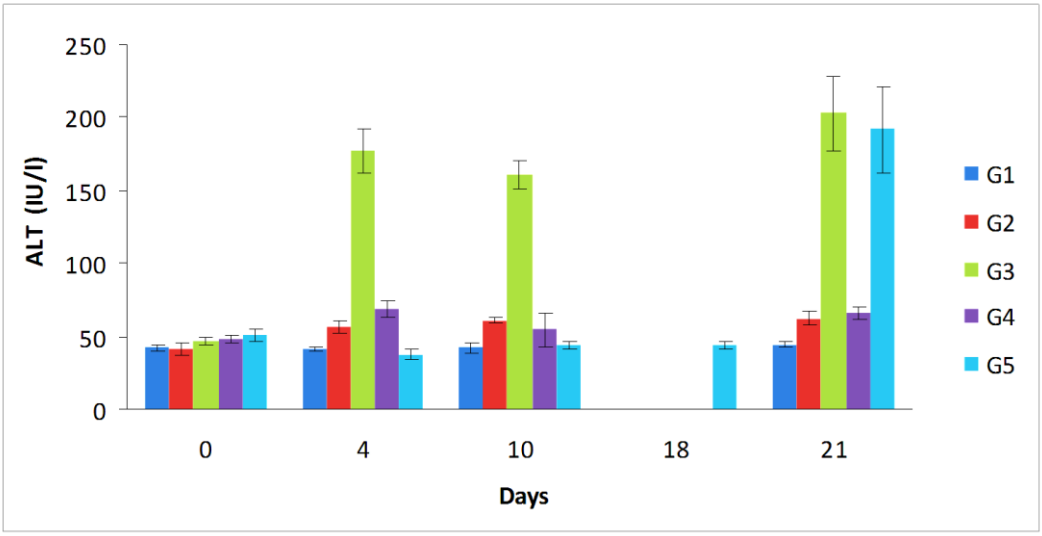
Serum AST activity of all the five groups of rats is presented in Table 7 and Fig. 7. The initial values recorded ranges from 123.14 ± 5.27 IU/l in G4 to 147.67 ± 9.75 IU/l in G1. On day four and day 10 of the experiment the rats in G3 group had a significantly (P≤ 0.05) increased AST activity when compared to all other groups. The G3 rats had an AST activity of 187.38 ± 9.16 IU/l, on day four and 187.43 ± 8.98, on day 10. On day four and 10, the G5 rats showed a significantly (P≤ 0.05) low AST activity (112.56 ± 8.75 IU/l and 113.22 ± 8.58 IU/l respectively) when compared to all other groups of rats. The other groups of rats (G1, G2 and G4) had a similar AST activity on both day four and day 10. On 21st day of the experiment, the rats in G3 and G5 had a significantly (P≤ 0.05) high AST activity (260.90 ± 13.24 IU/l and 227.01 ± 13.58 IU/l respectively).

Table 6. Effect of aqueous extract of Acerola berries on serum alanine amino transferase (ALT) activity of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	ALT (IU/l)				
	0	4	10	18	21
G1	42.24 ^{a}±1.82	41.28 ^{a}±1.65	42.19 ^{a}±3.12	-	44.02 ^{a} ±2.01
G2	40.78 ^{a}±3.93	56.31 ^{ab}±3.82	60.90 ^{b}±2.43	-	62.29 ^{a} ±4.43
G3	46.76 ^{a}±2.66	177.04 ^{c}*±15.2	160.65 ^{c}*±9.43	-	202.77 ^{b}*±25.90
G4	48.39 ^{a}±2.98	68.63 ^{b}±5.15	54.36 ^{ab}±11.19	-	65.52 ^{a} ±4.19
G5	50.94 ^{a}±4.42	37.59 ^{a}±2.96	43.50 ^{a}±2.58	44.12±2.72	191.97 ^{b}*±29.81

Values bearing different superscripts (a, b and c) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 6. Effect of aqueous extract of Acerola berries on serum alanine amino transferase (ALT) activity of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



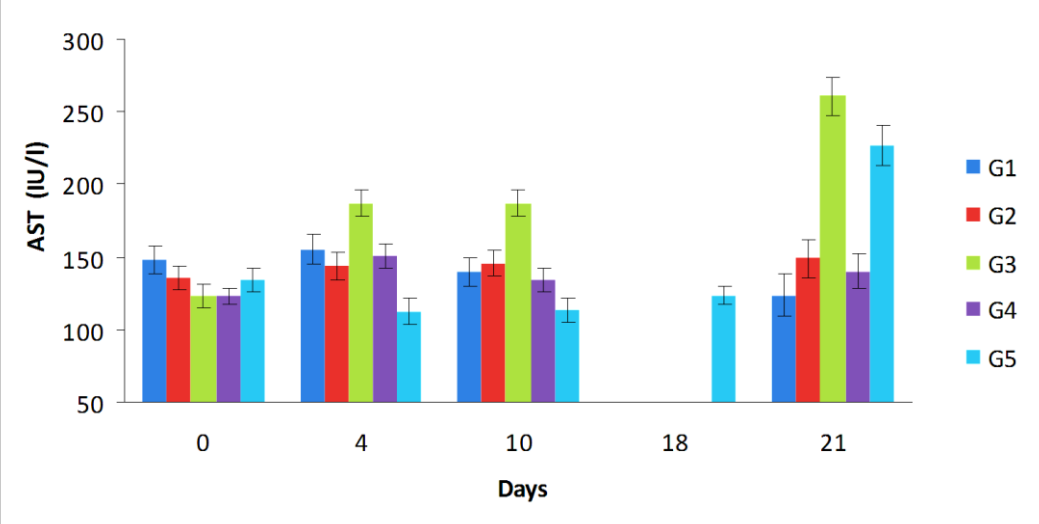
G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Table 7. Effect of aqueous extract of Acerola berries on serum aspartate amino transferase (AST) activity of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	AST (IU/l)				
	0	4	10	18	21
G1	147.67 ^{a}±9.75	155.49 ^{b}±10.01	139.95 ^{b}±9.81	-	123.54 ^{a} ±14.49^{a}
G2	135.69 ^{a}±8.50	144.21 ^{b}±9.27	145.49 ^{b}±9.09	-	149.16 ^{a} ±13.40^{a}
G3	123.24 ^{a}±7.92	187.38 ^{c}*±9.16	187.43 ^{c}*±8.98	-	260.90 ^{b}*±13.24
G4	123.14 ^{a}±5.27	151.22 ^{b}±8.57	134.04 ^{b}±8.40	-	140.46 ^{a} ±12.38^{a}
G5	134.10 ^{a}±8.12	112.56 ^{a}±8.75	113.22 ^{a}±8.58	123.67±6.20	227.01 ^{b}*±13.58

Values bearing different superscripts (a, b and c) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 7. Effect of aqueous extract of Acerola berries on serum aspartate amino transferase (AST) activity of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Throughout the experiment, the AST activity of G3 rats was significantly ($P \leq 0.05$) high when compared to initial activity. The AST activity in G5 rats was significantly ($P \leq 0.05$) higher on day 21, when compared to all other days of the experiment. In G1, G2 and G4 rats, no significant ($P > 0.05$) change in AST activity was observed on day four, 10 and 21 when compared to day zero of the experiment.

4.3.1.3 Alkaline Phosphatase (ALP)

The observations of serum ALP activity of different groups is listed in Table 8 and Fig. 8. Before the start of experiment (day zero), the rats in all the five groups had a similar ALP activity. However on day four, the ALP activity of G3 rats (1173.73 ± 35.63 IU/l) was significantly ($P \leq 0.05$) higher than all other groups of rats. The ALP activity of rats in the G2 (518.20 ± 34.35 IU/l), G4 (520.82 ± 31.75 IU/l), and G5 (538.36 ± 34.89 IU/l) groups were similar but were significantly ($P \leq 0.05$) high when compared to G1 rats (420.91 ± 35.57 IU/l).

On day 10 of the experiment, the serum ALP activity of G3 rats (1002.86 ± 39.71 IU/l), was significantly ($P \leq 0.05$) high when compared to all other groups. The rats in G2, G4 and G5 groups had a similar ALP activity of 546.79 ± 38.28 IU/l, 491.98 ± 35.38 IU/l and 546.13 ± 38.87 IU/l respectively. The ALP activity of G4 rats was also similar to that of G1 rats (425.50 ± 39.63 IU/l). On day 21, the G3 and G5 rats showed an ALP activity (1129.79 ± 31.96 IU/l and 986.33 ± 32.06 IU/l respectively) significantly ($P \leq 0.05$) higher than that of G1 (465.29 ± 31.93 IU/l), G2 (573.07 ± 31.59 IU/l) and G4 (500.11 ± 28.58 IU/l) rats. The G1 and G4 rats had a similar ALP activity.

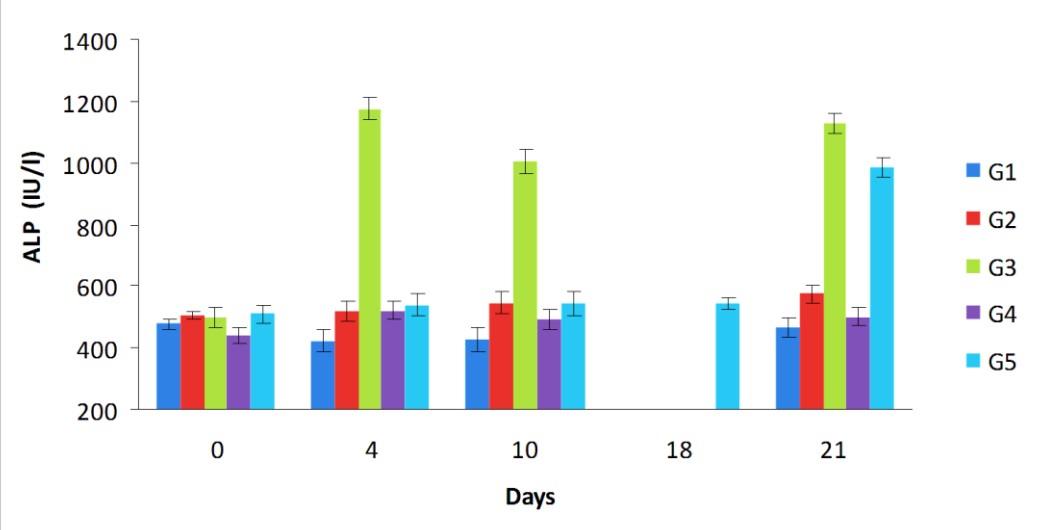
For the entire experimental period, the ALP activity of G3 rats was significantly ($P \leq 0.05$) high when compared to initial activity. In G5 rats, the ALP activity was significantly ($P \leq 0.05$) high on day 21 when compared to day zero. In

Table 8. Effect of aqueous extract of Acerola berries on serum alkaline phosphatase (ALP) activity of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	ALP (IU/l)				
	0	4	10	18	21
G1	476.47 [a] ±17.65	420.91 [a] ±35.57	425.50 [a] ±39.63	-	465.29 [a] ±31.93
G2	504.89 [a] ±13.98	518.20 [b] ±34.35	546.79 [b] ±38.28	-	573.07 [b] ±31.59
G3	498.74 [a] ±32.21	1173.73[c]*±35.63	1002.86[c]*±39.71	-	1129.79[c]*±31.96
G4	438.11 [a] ±25.07	520.82 [b] ±31.75	491.98 [ab] ±35.38	-	500.11 [a] ±28.58
G5	510.71 [a] ±28.68	538.36 [b] ±34.89	546.13 [b] ±38.87	545.83±19.02	986.33 [c]*±32.06

Values bearing different superscripts (a, b and c) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 8. Effect of aqueous extract of Acerola berries on serum serum alkaline phosphatase (ALP) activity (IU/l) of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

G1, G2 and G4 rats, and no significant (P> 0.05) change in ALP activity was observed through out whole experiment when compared to day zero.

4.3.2 Serum Protein Profile and A:G Ratio

4.3.2.1 Total Protein

Table 9 and Fig. 9 depict the mean value of serum total protein in different groups of rats. On day zero, the serum total protein levels were 4.40 ± 0.10 g/dl, 4.39 ± 0.14 g/dl, 4.17 ± 0.18 g/dl, 4.50 ± 0.20 g/dl and 4.22 ± 0.19 g/dl for groups G1, G2, G3, G4 and G5 respectively. Before paracetamol administration in G5 rats the mean value of serum total protein concentration observed on 18th day of the experiment was 4.58 ± 0.13 g/dl. On day four, the serum total protein concentration ranged from 4.24 g/dl in G1 and G5 rats to 4.52 g/dl in G4 rats. On day 10 the maximum serum total protein level was 4.51 g/dl in G5 rats to 4.12 g/dl in G3 rats.

On the final day of the experiment, serum total protein level was between 4.17 g/dl in G3 rats and 4.59 g/dl in G4 rats. However, no significant (P> 0.05) variation in serum total protein concentration was noticed between the different groups throughout the experiment. Similarly, when compared to day zero, no significant (P> 0.05) changes in the total protein level was observed in all the group of rats on day four, 10 and 21 of the experiment.

4.3.2.2 Albumin

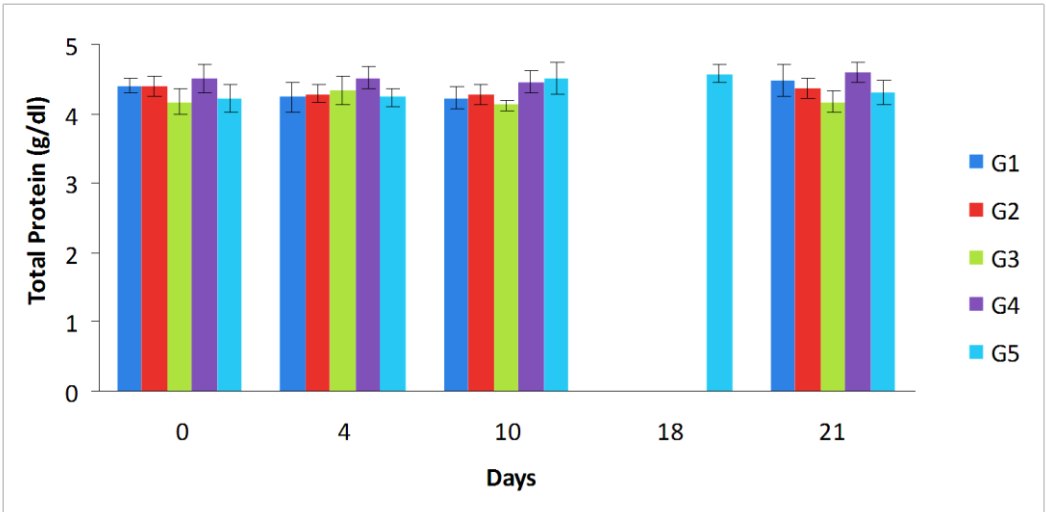
The level of serum albumin recorded at different stages of the study is presented in Table 10 and Fig. 10. There was no significant (P> 0.05) variation in serum albumin concentration noticed between all the five groups during the entire experiment. On day 21 the rats in the paracetamol administered group, G3 (2.42 ± 0.17 g/dl) showed the least serum albumin concentration. In all the groups, no

Table 9. Effect of aqueous extract of Acerola berries on serum total protein level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Total Protein (g/dl)				
	0	4	10	18	21
G1	4.40 [a]±0.10	4.24 [a]±0.21	4.227 [a] ±0.16	-	4.48 [a] ±0.23
G2	4.39 [a]±0.14	4.29 [a]±0.14	4.280 [a] ±0.14	-	4.36 [a] ±0.14
G3	4.17 [a]±0.18	4.34 [a]±0.20	4.123 [a] ±0.07	-	4.17 [a] ±0.16
G4	4.50 [a]±0.20	4.52 [a]±0.17	4.461 [a] ±0.15	-	4.59 [a] ±0.14
G5	4.22 [a]±0.19	4.24 [a]±0.13	4.512 [a] ±0.22	4.58±0.13	4.31 [a] ±0.18

Values bearing a common superscript (a) in columns did not differ significantly (P> 0.05) between groups.

Fig. 9. Effect of aqueous extract of Acerola berries on serum total protein level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



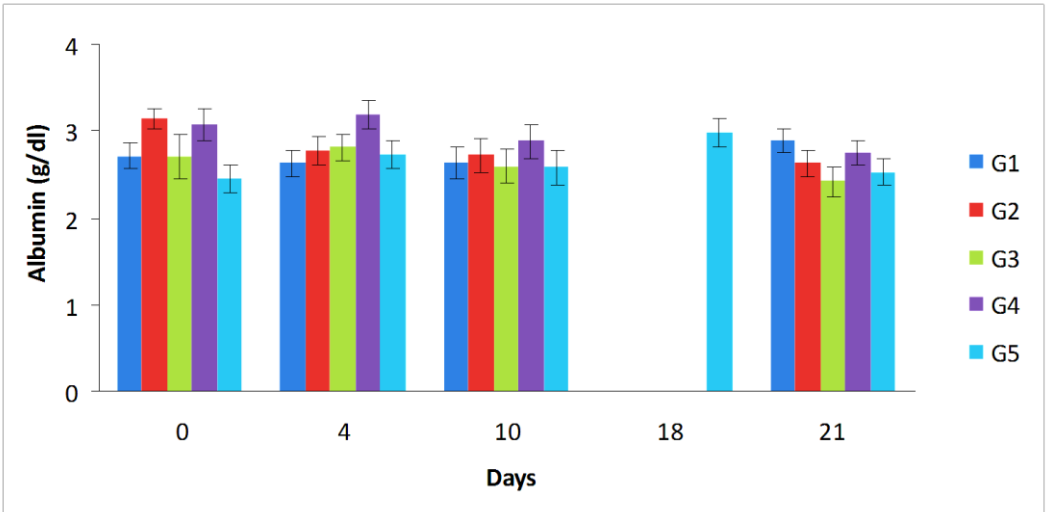
G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Table 10. Effect of aqueous extract of Acerola berries on serum albumin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Albumin (g/dl)				
	0	4	10	18	21
G1	2.71 [a] ±0.15	2.63 [a] ±0.15	2.64 [a] ±0.18	-	2.90 [a] ±0.14
G2	3.14 [a] ±0.12	2.78 [a] ±0.16	2.72 [a] ±0.19	-	2.63 [a] ±0.15
G3	2.71 [a] ±0.25	2.81 [a] ±0.15	2.60 [a] ±0.19	-	2.42 [a] ±0.17
G4	3.08 [a] ±0.18	3.20 [a] ±0.16	2.88 [a] ±0.19	-	2.75 [a] ±0.14
G5	2.45 [a] ±0.17	2.72 [a] ±0.16	2.58 [a] ±0.19	2.98±0.17	2.53 [a] ±0.16

Values bearing a common superscript (a) in columns did not differ significantly (P> 0.05) between groups.

Fig. 10. Effect of aqueous extract of Acerola berries on serum albumin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

significant (P> 0.05) difference in serum albumin level was recorded between the different days studied, when compared to the initial concentration.

4.3.2.3 Globulin

The mean serum globulin levels of different groups of rats (G1 to G5) are presented in Table 11 and Fig. 11. On day zero, the observed serum globulin concentration were 1.69 ± 0.23 g/dl, 1.25 ± 0.11 g/dl, 1.42 ± 0.18 g/dl, 1.42 ± 0.14 g/dl and 1.52 ± 0.13 g/dl for the groups, G1, G2, G3, G4 and G5 respectively. During the entire experiment, the serum globulin concentration did not differ significantly (P> 0.05) between the groups. When compared to day zero, an insignificant (P> 0.05) increase in serum globulin level was noticed in paracetamol administered (G3) group of rats (1.63 ± 0.16 and 1.65 ± 0.11 g/dl) on day 10 and 21 respectively. Also an insignificant (P> 0.05) decrease in serum globulin concentration was observed on day 21 in the Acerola treated, G4 group of rats (1.37 ± 0.16 g/dl) when compared to the initial value.

4.3.2.4 Albumin: Globulin (A:G) Ratio

The serum A:G ratio of the control and experimental group of rats is shown in Table 12 and Fig. 12. The mean A:G ratio noticed did not differ significantly (P> 0.05) between all the different groups of rats throughout the entire experiment (from day zero to day 21). However, a decreased ratio (insignificant) was recorded in G3 (1.86 ± 0.22) and G5 (1.97 ± 0.19) group of rats on day 21.

4.3.3 Serum Total Cholesterol

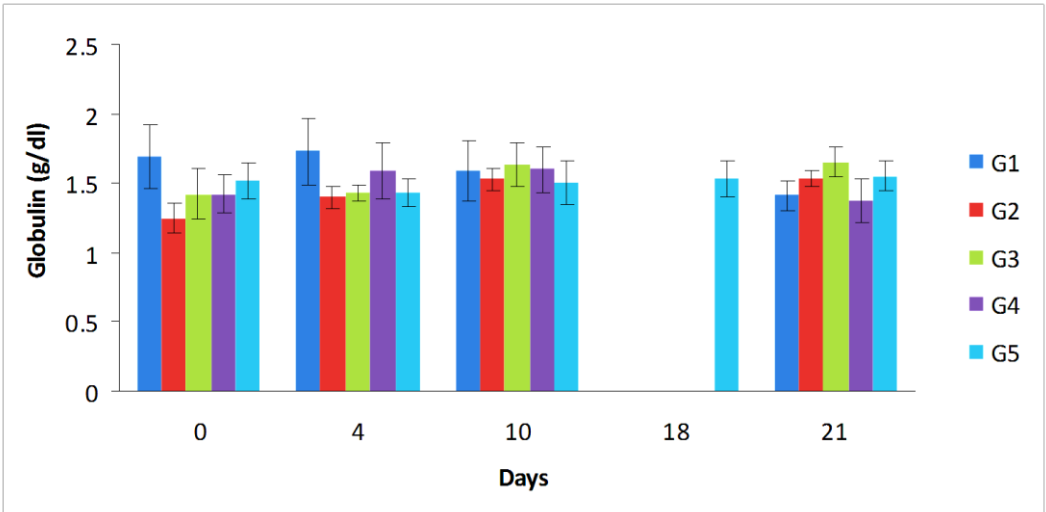
The serum total cholesterol concentration obtained in different groups is presented in Table 13 and Fig. 13. Mean data obtained for serum total cholesterol on day zero were 40.91 ± 4.03 mg/dl, 39.89 ± 5.76 mg/dl, 37.59 ± 2.99 mg/dl,

Table 11. Effect of aqueous extract of Acerola berries on serum globulin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Globulin (g/dl)				
	0	4	10	18	21
G1	1.69 [a] ±0.23	1.73 [a] ±0.24	1.59 [a] ±0.22	-	1.41 [a] ±0.11
G2	1.25 [a] ±0.11	1.40 [a] ±0.08	1.53 [a] ±0.08	-	1.53 [a] ±0.06
G3	1.42 [a] ±0.18	1.43 [a] ±0.06	1.63 [a] ±0.16	-	1.65 [a] ±0.11
G4	1.42 [a] ±0.14	1.59 [a] ±0.20	1.60 [a] ±0.17	-	1.37 [a] ±0.16
G5	1.52 [a] ±0.13	1.43 [a] ±0.10	1.50 [a] ±0.16	1.53±0.13	1.55 [a] ±0.11

Values bearing a common superscript (a) in columns did not differ significantly (P> 0.05) between groups.

Fig. 11. Effect of aqueous extract of Acerola berries on serum globulin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



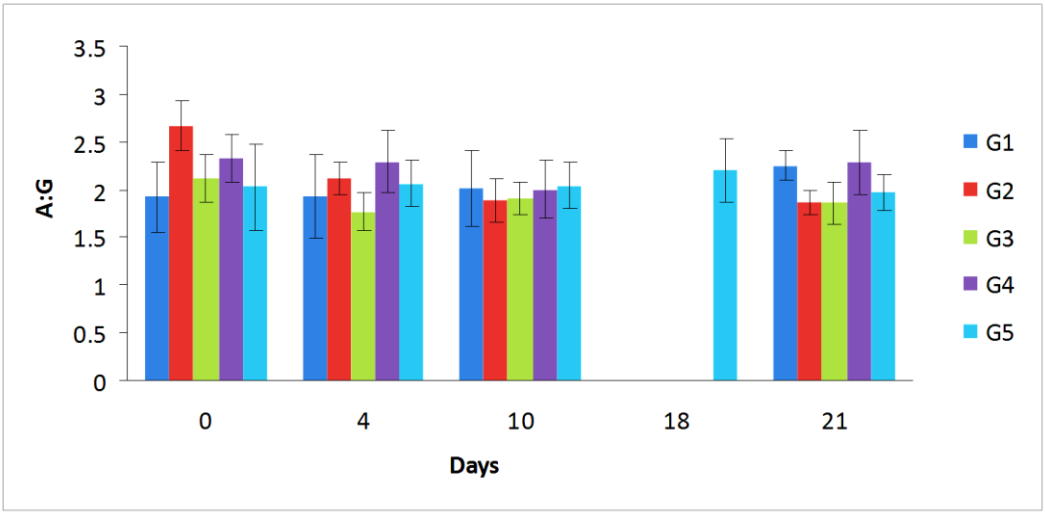
G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Table 12. Effect of aqueous extract of Acerola berries on serum albumin: globulin (A:G) ratio of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	A:G ratio				
	0	4	10	18	21
G1	1.92 [a] ±0.36	1.92 [a] ±0.44	2.01 [a] ±0.39	-	2.25 [a] ±0.16
G2	2.67 [a] ±0.27	2.11 [a] ±0.17	1.89 [a] ±0.23	-	1.87 [a] ±0.12
G3	2.12 [a] ±0.25	1.77 [a] ±0.20	1.91 [a] ±0.16	-	1.86 [a] ±0.22
G4	2.32 [a] ±0.25	2.29 [a] ±0.32	2.00 [a] ±0.31	-	2.29 [a] ±0.34
G5	2.03 [a] ±0.45	2.06 [a] ±0.24	2.04 [a] ±0.24	2.20±0.33	1.97 [a] ±0.19

Values bearing a common superscript (a) in columns did not differ significantly (P> 0.05) between groups.

Fig. 12. Effect of aqueous extract of Acerola berries on serum albumin: globulin (A:G) ratio of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

43.13 ± 5.36 mg/dl and 46.60 ± 3.21 mg/dl for G1, G2, G3, G4 and G5 groups respectively. There was no significant (P> 0.05) difference in the initial (day zero) serum total cholesterol level of the rats in different groups.

On day four, there was no significant (P> 0.05) difference in serum total cholesterol concentration of rats in G1 (57.68 ± 5.09 mg/dl), G2 (68.01 ± 4.40 mg/dl), G3 (62.65 ± 5.37 mg/dl) and G4 (57.63 ± 4.40 mg/dl). But the G2 and G3 rats had a significantly (P≤ 0.05) high cholesterol concentration when compared to G5 rats protected with Acerola. The Acerola fed G5 rats showed the least cholesterol level on day four (43.66 ± 3.94 mg/dl) and day 10 (43.72 ± 5.29 mg/dl), though they have the highest concentration on day zero.

On 10th day of the study, the G3 rats showed a significantly (P≤ 0.05) high serum total cholesterol concentration of 74.53 ± 4.79 mg/dl when compared to G1 (55.72 ± 5.25 mg/dl), G2 (50.72 ± 6.73 mg/dl) and G5 (43.72 ± 5.29 mg/dl) rats. The G4 rats had a cholesterol concentration of 65.16 ± 7.50 mg/dl similar to G1, G2 and G3, but significantly (P≤ 0.05) high when compared to the cholesterol concentration of G5 rats. On day 21, the serum total cholesterol concentration recorded in G3 rats (104.53 ± 7.59 mg/dl) was significantly (P≤ 0.05) high when compared to G1 (44.90 ± 6.38 mg/dl), G2 (50.44 ± 6.62 mg/dl) and G4 (76.46 ± 5.92 mg/dl) rats. The G5 rats had a significantly (P≤ 0.05) high cholesterol concentration (98.18 ± 12.41 mg/dl) when compared to G1 and G2.

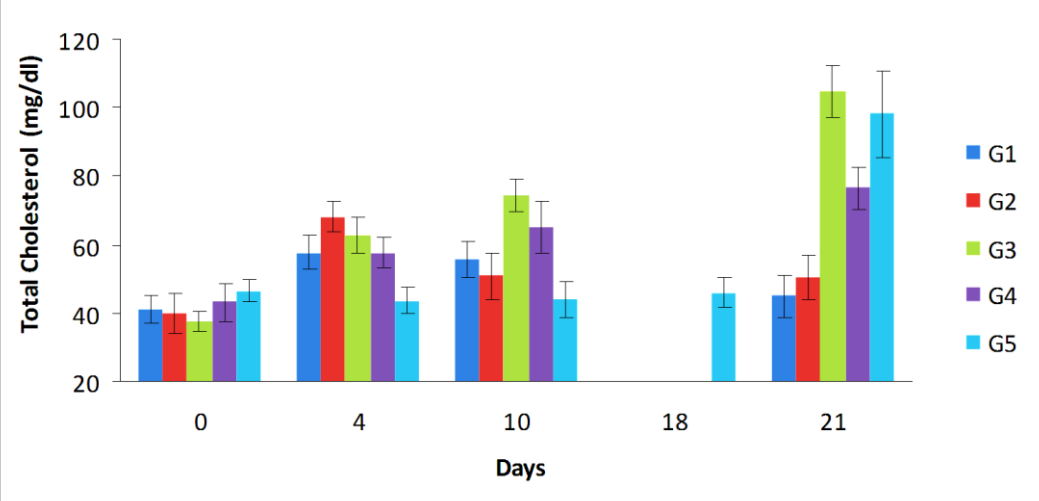
Throughout the experiment, the serum total cholesterol concentration of G3 rats was significantly (P≤ 0.05) high when compared to initial cholesterol concentration. In G4 rats also, significantly (P≤ 0.05) high cholesterol concentration was noticed on day 10 and 21 when compared to value on day zero. On 21st day of the experiment, the cholesterol concentration of G5 rats showed a significantly (P≤ 0.05) elevated value when compared to the initial cholesterol concentration of the same group.

Table 13. Effect of aqueous extract of Acerola berries on serum total cholesterol concentration of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Total Cholesterol (mg/dl)				
	0	4	10	18	21
G1	40.91 ^{a}±4.03	57.68 ^{ab}±5.09	55.72 ^{ab} ±5.25	-	44.90 ^{a} ±6.38
G2	39.89 ^{a}±5.76	68.01^{b}*±4.40	50.72 ^{ab} ±6.73	-	50.44 ^{a} ±6.62
G3	37.59 ^{a}±2.99	62.65^{b}*±5.37	74.53 ^{c}*±4.79	-	104.53 ^{c}*±7.59
G4	43.13 ^{a}±5.36	57.63 ^{ab}±4.40	65.16 ^{b} ±7.50	-	76.46 ^{b}*±5.92
G5	46.60 ^{a}±3.21	43.66 ^{a} ±3.94	43.72 ^{a} ±5.29	45.93±4.27	98.18 ^{bc}*±12.41

Values bearing different superscripts (a, b and c) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 13. Effect of aqueous extract of Acerola berries on serum total cholesterol concentration of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

4.3.4 Blood Urea Nitrogen (BUN)

The observed mean values of BUN level of the control and experimental groups of rats are listed in Table 14 and Fig. 14. On the initial day of experiment, the observed BUN levels for G1, G2, G3, G4 and G5 group of rats were 28.75 ± 2.55 mg/dl, 30.63 ± 4.57 mg/dl, 29.29 ± 2.54 mg/dl and 34.38 ± 3.20 mg/dl respectively. No significant (P> 0.05) deviation was observed on day zero in the BUN level values of different groups of rats. In paracetamol administered group of rats G3, on day four and 10, significant (P≤ 0.05) increase in BUN level (90.71 ± 12.41 mg/dl and 85.71 ± 13.86 mg/dl respectively) was observed. Considerable changes were not observed in BUN concentration in any groups of rats.

On day 21, the G3 and G5 rats showed significantly (P≤ 0.05) elevated BUN level (98.57 ± 15.30 mg/dl and 79.29 ± 9.79 mg/dl respectively) when compared with G1 (27.50 ± 3.27 mg/dl), G2 (33.13 ± 4.32 mg/dl) and G4 (28.75 ± 4.70 mg/dl). On day four, 10 and 21 of the experiment, G3 rats showed a significantly (P≤ 0.05) higher BUN level when compared to initial value. On day 21 the G5 group of rats also showed a significantly (P≤ 0.05) elevated BUN level when compared to day zero BUN value.

4.3.5 Serum Total, Direct and Indirect Bilirubin

4.3.5.1 Total Bilirubin

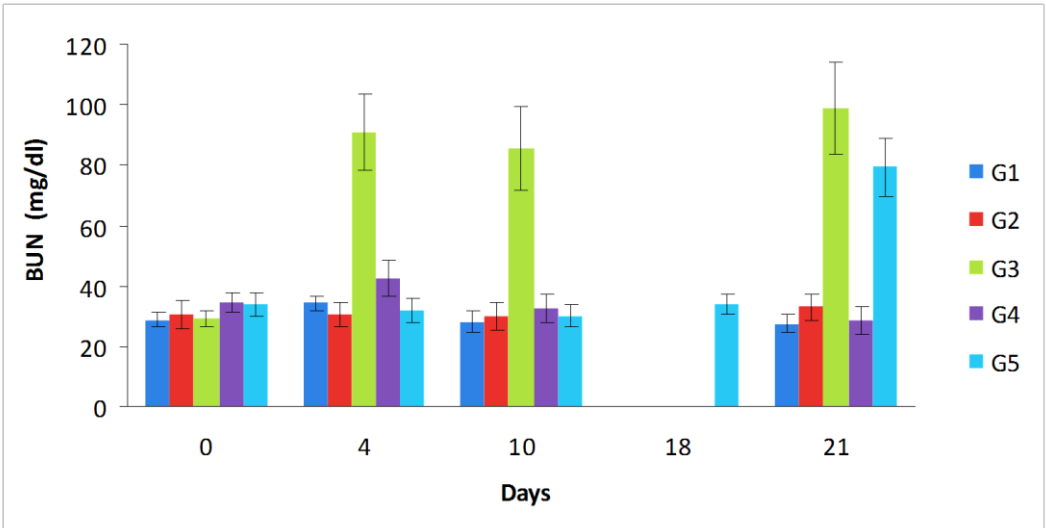
Serum total bilirubin levels observed in all the groups of rats are presented in Table 15a and Fig. 15a. The preliminary mean observation of the five experimental groups were 0.74 ± 0.05 mg/dl, 0.73 ± 0.06 mg/dl, 0.74 ± 0.04 mg/dl, 0.67 ± 0.05 mg/dl and 0.73 ± 0.06 mg/dl respectively. The serum total bilirubin concentration was significantly (P≤ 0.05) high in G3 rats and this increase was noticed on day four (1.14 ± 0.05 mg/dl), day 10 (1.05 ± 0.05 mg/dl) and day 21 (1.11 ± 0.05 mg/dl).

Table 14. Effect of aqueous extract of Acerola berries on blood urea nitrogen (BUN) level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	BUN (mg/dl)				
	0	4	10	18	21
G1	28.75 [a] ±2.46	34.39 [a] ±2.40	28.13 [a] ±3.40	-	27.50 [a] ±3.27
G2	30.63 [a] ±4.58	30.63 [a] ±4.17	30.00 [a] ±4.53	-	33.13 [a] ±4.32
G3	29.29 [a] ±2.54	90.71 [b]*±12.41	85.71 [b]*±13.86	-	98.57 [b]*±15.30
G4	34.38 [a] ±3.20	42.50 [a] ±5.83	32.50 [a] ±4.63	-	28.75 [a]±4.70
G5	33.75 [a] ±4.20	31.88 [a] ±3.89	30.00 [a] ±3.78	33.75±3.24	79.29 [b]*±9.79

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 14. Effect of aqueous extract of Acerola berries on blood urea nitrogen (BUN) level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

In G1, G2 and G4 rats, throughout the whole experiment no significant (P> 0.05) changes in serum total bilirubin level were observed between groups. Similarly these groups of rats did not show any significant (P> 0.05) change in serum total bilirubin concentration on day four, 10 and 21 when compared to day zero. After paracetamol administration in G5 rats, a considerable (P≤ 0.05) elevation in serum total bilirubin concentration was observed on the 21st day (1.26 ± 0.09 mg/dl), when compared to day zero. The bilirubin concentration recorded in G3 and G5 rats on day 21 did not vary significantly (P> 0.05).

4.3.5.2 Direct Bilirubin

The mean data for serum direct bilirubin concentration of each group is listed in Table 15b and Fig. 15b. On day zero, no significant (P> 0.05) difference was observed in direct bilirubin levels all the five groups. As in case of total bilirubin, throughout the experiment the direct bilirubin concentration of paracetamol administered group of rats (G3), was significantly (P≤ 0.05) high when compared to all other groups of rats. No significant (P> 0.05) changes were observed in serum direct bilirubin concentration between G1, G2 and G4 group of rats for the entire period of the experiment.

The G5 rats recorded significantly (P≤ 0.05) elevated serum direct bilirubin value on day 21 (0.98 ± 0.05 mg/dl) that is, two days after the induction of paracetamol toxicity. Prior to paracetamol administration (18th day), the mean direct bilirubin concentration observed in G5 rats was 0.58 ± 0.08 mg/dl.

4.3.5.3 Indirect Bilirubin

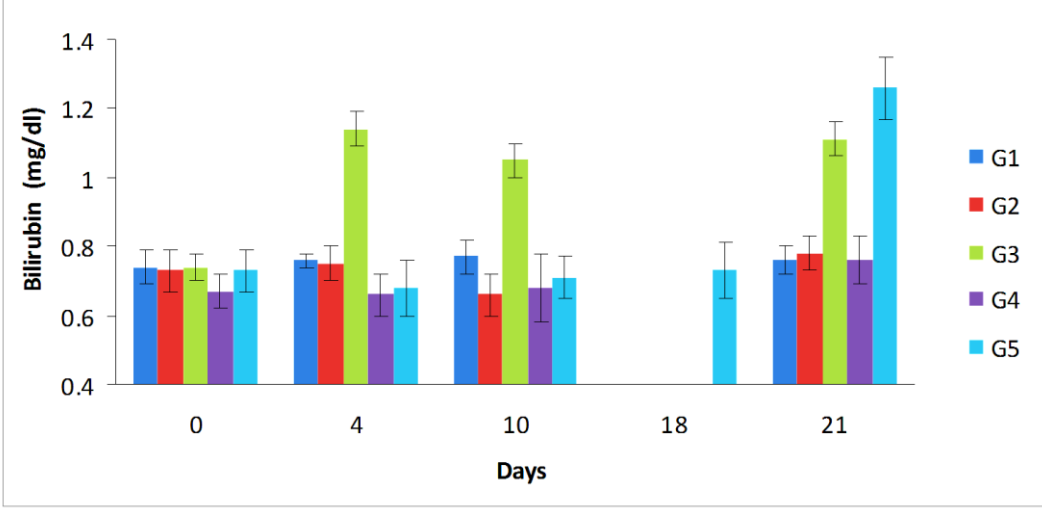
The recorded mean value of serum indirect bilirubin is shown in Table 15c and Fig. 15c. The initial values of indirect bilirubin recorded on day zero did not show any significant (P> 0.05) variation between the different groups of rats. The observations for indirect bilirubin in paracetamol induced hepatotoxic group of

Table 15a. Effect of aqueous extract of Acerola berries on serum total bilirubin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Total Bilirubin (mg/dl)				
	0	4	10	18	21
G1	0.74 [a] ±0.05	0.76 [a] ±0.02	0.77 [a] ±0.05	-	0.76 [a] ±0.04
G2	0.73 [a] ±0.06	0.75 [a] ±0.05	0.66 [a] ±0.06	-	0.78 [a] ±0.05
G3	0.74 [a] ±0.04	1.14 [b]*±0.05	1.05 [b]*±0.05	-	1.11 [b]*±0.05
G4	0.67 [a] ±0.05	0.66 [a] ±0.06	0.68 [a] ±0.10	-	0.76 [a] ±0.07
G5	0.73 [a] ±0.06	0.68 [a] ±0.08	0.71 [a] ±0.06	0.73±0.08	1.26 [b]*±0.09

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 15a. Effect of aqueous extract of Acerola berries on serum total bilirubin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



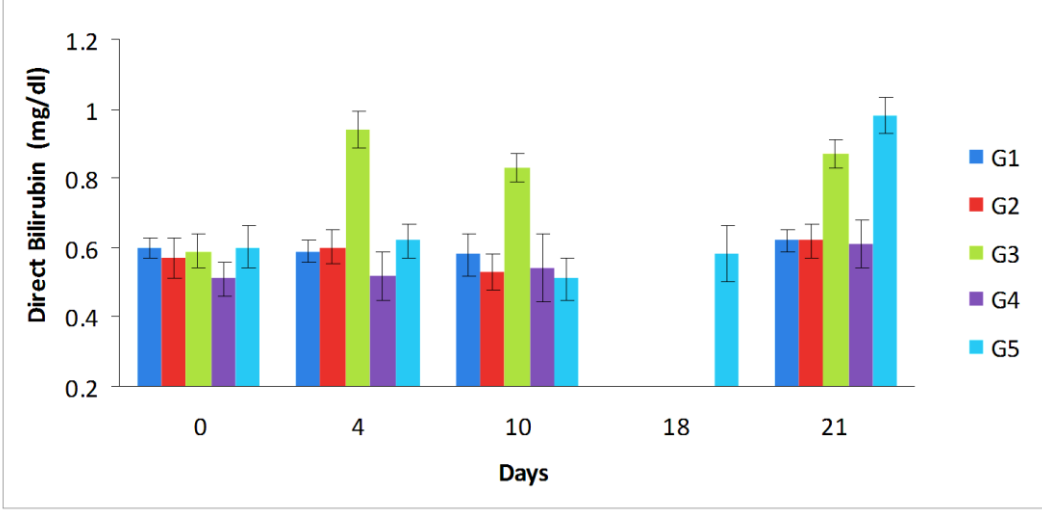
G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Table 15b. Effect of aqueous extract of Acerola berries on serum direct bilirubin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Direct Bilirubin (mg/dl)				
	0	4	10	18	21
G1	0.60[a]±0.03	0.59 [a] ±0.03	0.58 [a] ±0.06	-	0.62 [a] ±0.03
G2	0.57[a]±0.06	0.60 [a] ±0.05	0.53 [a] ±0.05	-	0.62 [a] ±0.05
G3	0.59[a]±0.05	0.94 [b]*±0.05	0.83 [b]*±0.04	-	0.87 [b]*±0.04
G4	0.51[a]±0.05	0.52 [a] ±0.07	0.54 [a] ±0.10	-	0.61 [a] ±0.07
G5	0.60[a]±0.06	0.62 [a] ±0.05	0.51 [a] ±0.06	0.58±0.08	0.98 [b]*±0.05

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 15b. Effect of aqueous extract of Acerola berries on serum direct bilirubin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



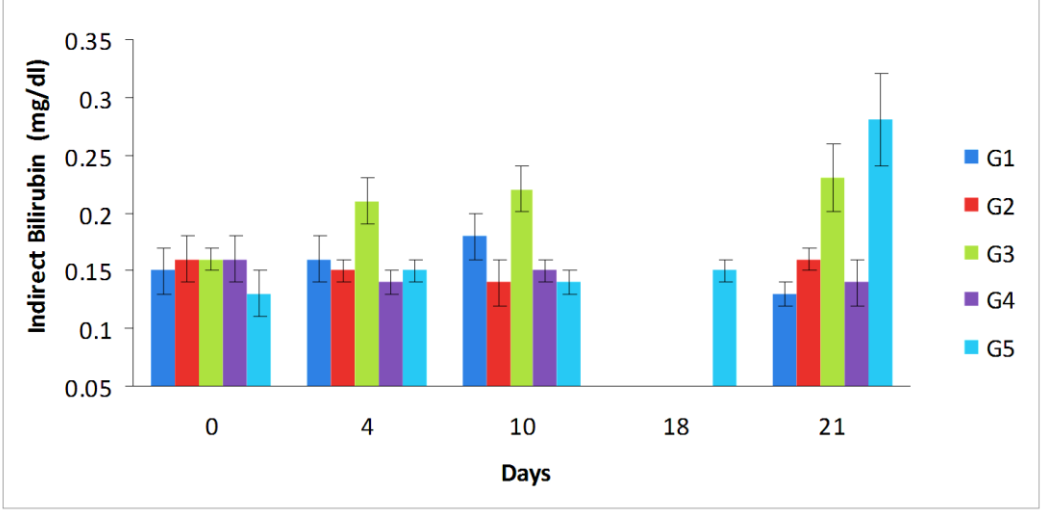
G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Table 15c. Effect of aqueous extract of Acerola berries on serum indirect bilirubin level of paracetamol induced hepatotoxic rats (Mean ± SE, n =8).

Days / Groups	Indirect Bilirubin (mg/dl)				
	0	4	10	18	21
G1	0.15[a]±0.02	0.16 [ab] ±0.02	0.18 [ab] ±0.02	-	0.13 [a] ±0.01
G2	0.16[a]±0.02	0.15 [a] ±0.01	0.14 [a] ±0.02	-	0.16 [a] ±0.01
G3	0.16[a]±0.01	0.21 [b]*±0.02	0.22 [b]*±0.02	-	0.23 [b]*±0.03
G4	0.16[a]±0.02	0.14 [a] ±0.01	0.15 [a] ±0.01	-	0.14 [a] ±0.02
G5	0.13[a]±0.02	0.15 [a] ±0.01	0.14 [a] ±0.01	0.15±0.01	0.28 [b]*±0.04

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st.

Fig. 15c. Effect of aqueous extract of Acerola berries on serum indirect bilirubin level of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

rats, G3 were 0.21 ± 0.02 mg/dl, 0.22 ± 0.02 mg/dl and 0.23 ± 0.03 mg/dl on day four, 10 and 21 respectively. There was a significant (P≤ 0.05) elevation of serum indirect bilirubin level observed in G3 rats, when compared to G1, G2 and G4 group of rats. No significant (P> 0.05) change indirect bilirubin concentration was noticed between G1, G2, G4 and G5 group of rats on day four and 10.

When compared to all other groups (G1, G2 and G4) of rats the indirect bilirubin levels of G5 rats was significantly (P≤ 0.05) elevated on day 21 (0.28 ± 0.04 mg/dl). No substantial changes were found between all other groups. On day four, 10 and 21 of the experiment, G3 rats showed a significantly (P≤ 0.05) higher serum indirect bilirubin concentration when compared to initial zero day value. On day 21, the level of serum indirect bilirubin recorded in G5 group of rats also was significantly (P≤ 0.05) elevated level when compared to initial value.

4.3.6 Antioxidant Profile

4.3.6.1 Serum Reduced Glutathione

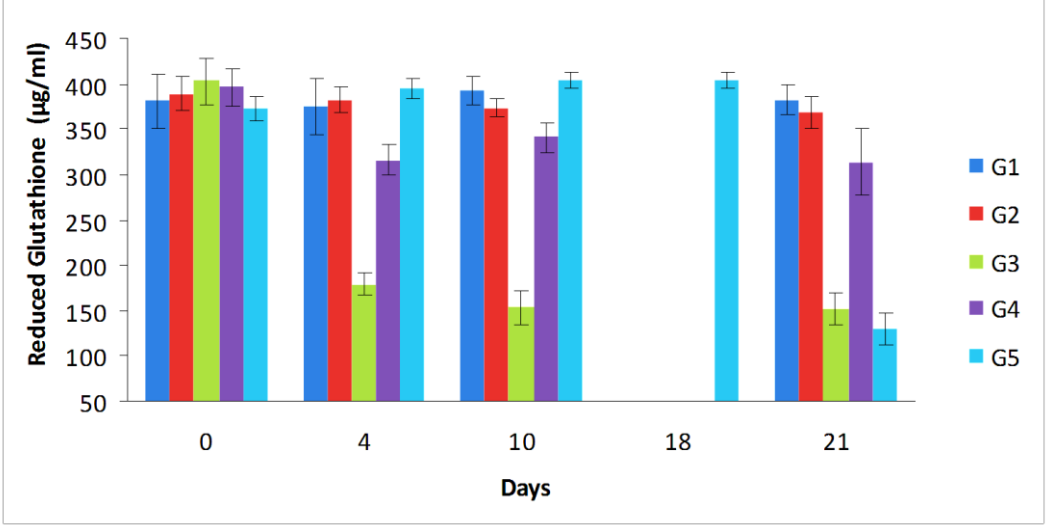
Serum reduced glutathione content of all the five experimental groups on day zero, four, 10 and 21 are presented in Table 16 and Fig. 16. On initial day of the experiment, there was no significant (P> 0.05) change in serum reduced glutathione content between all the groups of rats. The rats in G3 group had the lowest serum reduced glutathione content throughout the experimental period. On fourth day, the G4 group of rats, supplemented with Acerola along with paracetamol had a significantly (P≤ 0.05) less (316.25 ± 16.68 µg/ml) reduced glutathione content, when compared to G1 (375.00 ± 30.76 µg/ml), G2 (382.50 ± 14.73 µg/ml) and G5 (395.00 ± 10.69 µg/ml) rats, but was significantly (P≤ 0.05) high when compared to paracetamol administered group of rats, G3 (178.57 ± 12.43 µg/ml). On day 10, significantly (P≤ 0.05) low serum reduced glutathione activity was recorded in G3 rats (152.86 ± 19.24 µg/ml), when compared to all

Table 16. Effect of aqueous extract of Acerola berries on serum reduced glutathione content of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Days / Groups	Reduced Glutathione (µg/ml)				
	0	4	10	18	21
G1	381.25^{a}±29.60	375.00^{c} ±30.76	392.50^{c} ±16.45	-	382.50^{b} ±17.40
G2	388.75^{a}±18.94	382.50^{c} ±14.73	373.75^{c} ±10.22	-	368.75^{b} ±17.16
G3	402.86^{a}±24.76	178.57^{a}*±12.43	152.86^{a}*±19.24	-	151.43^{a}*±18.31
G4	396.25^{a}±20.95	316.25^{b}*±16.68	341.26^{b}*±16.95	-	313.75^{b}*±36.29
G5	372.50^{a}±12.92	395.00^{c} ±10.69	403.75^{c} ±8.22	403.75±9.10	130.00^{a}*±18.25

Values bearing different superscripts (a, b and c) in columns differ significantly (P≤ 0.05) between groups. Means bearing * in rows indicate significant (P≤ 0.05) difference between day zero and respective days, fourth, 10th and 21st

Fig. 16. Effect of aqueous extract of Acerola berries on serum reduced glutathione content of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

other groups of rats. The G4 rats showed (341.26±16.95 µg/ml) a significantly ($P \leq 0.05$) less value, when compared to G1 (392.50 ± 16.45 µg/ml), G2 (373.75 ± 10.22 µg/ml) and G5 (403.75 ± 8.22 µg/ml) rats. On 21st day of the experiment, G3 (151.43 ± 18.31 µg/ml) and G5 (130.00 ± 18.25 µg/ml) rats had a significantly ($P \leq 0.05$) low concentration of serum reduced glutathione when compared to rest of the groups.

The G3 and G4 rats showed a significantly ($P \leq 0.05$) less serum reduced glutathione content throughout the experiment when compared to the initial day value. On day 21, the reduced glutathione content of G5 rats was significantly ($P \leq 0.05$) less when compared to day zero.

4.3.6.2 Liver Reduced Glutathione

The mean values obtained for liver reduced glutathione content are shown in Table 17 and Fig. 17a. On the last day of experiment, the liver reduced glutathione content was significantly ($P \leq 0.05$) less in G3 (1092.86 ± 16.72 µg/g) and G5 (1124.29 ± 35.31 µg/g) rats, when compared to G1 (1446.25 ± 17.11 µg/g), G2 (1406.25 ± 15.35 µg/g) and G4 (1382.50 ± 15.44 µg/g) group of rats.

4.3.6.3 Lipid Peroxides in Liver

The data observed for liver lipid peroxide content are presented in Table 17 and Fig. 17b. The level of lipid peroxides in liver at the end of experiment in G3 (659.14 ± 47.31 nM/g) and G5 (850.29 ± 83.98 nM/g) rats were significantly ($P \leq 0.05$) elevated when compared with G1 (355.25 ± 14.78 nM/g), G2 (368.13 ± 15.82 nM/g) and G4 (401.50 ± 22.14 nM/g) group of rats.

4.3.6.4 Superoxide dismutase (SOD) in Liver

The observations of liver SOD activity are presented in the Table 17 and Fig. 17c. The mean normal SOD activity was found to be 49.26 ± 1.42 U/mg of protein. Out of five groups the SOD activity was lowest (23.58 ± 1.53 U/mg of protein) for G3 rats (paracetamol alone). A significant ($P \leq 0.05$) increase in the SOD activity was noted in the G4 group (42.26 ± 1.87 U/mg of protein) when compared with paracetamol induced hepatotoxic group. The SOD activity of G5 rats (26.19 ± 2.06 U/mg of protein) was similar to that of paracetamol alone administered group of rats. There was no significant ($P > 0.05$) difference between G1 and G2 (48.45 ± 1.67 U/mg of protein) rats.

4.4 HISTOPATHOLOGICAL EXAMINATION

4.4.1 Liver

Histology of liver from the control (G1) group revealed normal hepatic architecture showing radiating hepatocytes with vesicular nuclei around the central vein. Portal triad and sinusoidal spaces remained normal (Plate-3). The liver section from vehicle administered (G2) group also showed normal structure (Plate-4).

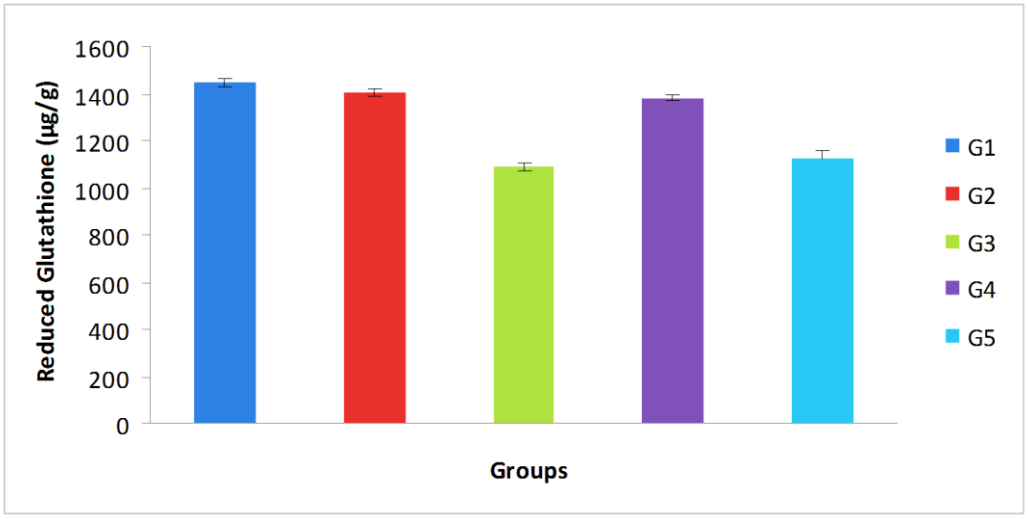
On day four of the experiment, there was coagulation necrosis and severe haemorrhage in the hepatic lobules of G3 rats (Plate-5). However necrotic lesions were less severe when compared to day 21 (Plate-5 and 6). On day 21, there was severe degeneration of hepatic cells with intense centrilobular necrosis extending to mid-zone (Plate-6) with sinusoidal haemorrhages and dilatation. There were mononuclear cell infiltration in the portal tracts, extensive areas of coagulation necrosis, multifocal diffuse haemorrhage, cloudy swelling and eosinophilic cytoplasm.

Table 17. Effect of aqueous extract of Acerola berries on liver reduced glutathione content, lipid peroxide level and superoxide dismutase (SOD) activity of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

Groups	Liver Reduced Glutathione (µg/g)	Lipid Peroxides in liver (nM/g)	Liver SOD (U/mg of protein)
G1	1446.25 ^{b} ±17.11	355.25 ^{a} ±14.78	49.26 ^{b} ±1.42
G2	1406.25 ^{b} ±15.35	368.13 ^{a} ±15.82	48.45 ^{b} ±1.67
G3	1092.86 ^{a} ±16.72	659.14 ^{b} ±47.31	23.58 ^{a} ±1.53
G4	1382.50 ^{b} ±15.44	401.50 ^{a} ±22.14	42.26 ^{b} ±1.87
G5	1124.29 ^{a} ±35.31	850.29 ^{b} ±83.98	26.19 ^{a} ±2.06

Values bearing different superscripts (a and b) in columns differ significantly (P≤ 0.05) between groups.

Fig. 17a. Effect of aqueous extract of Acerola berries on liver reduced glutathione content of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19.

Fig. 17b. Effect of aqueous extract of Acerola berries on level of liver lipid peroxides of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).

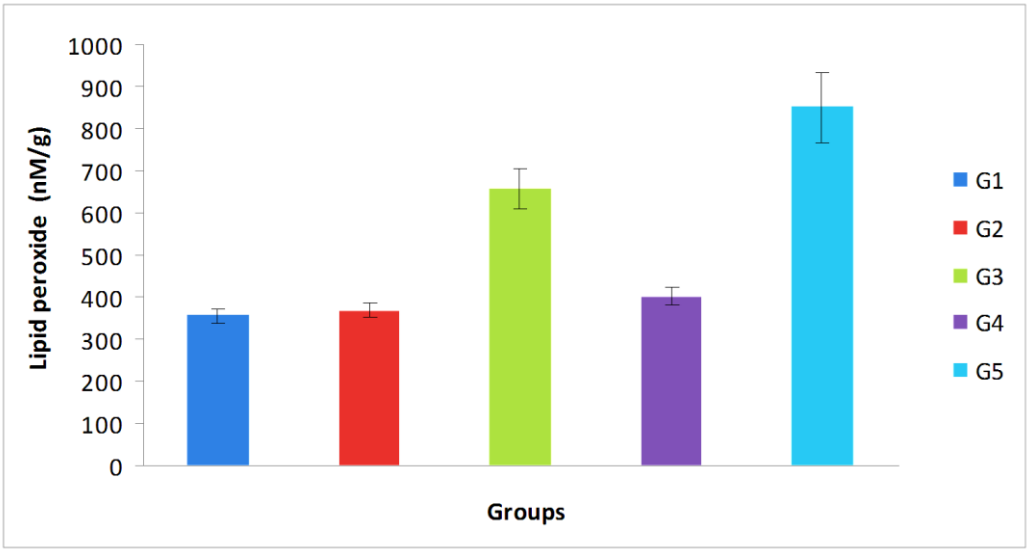
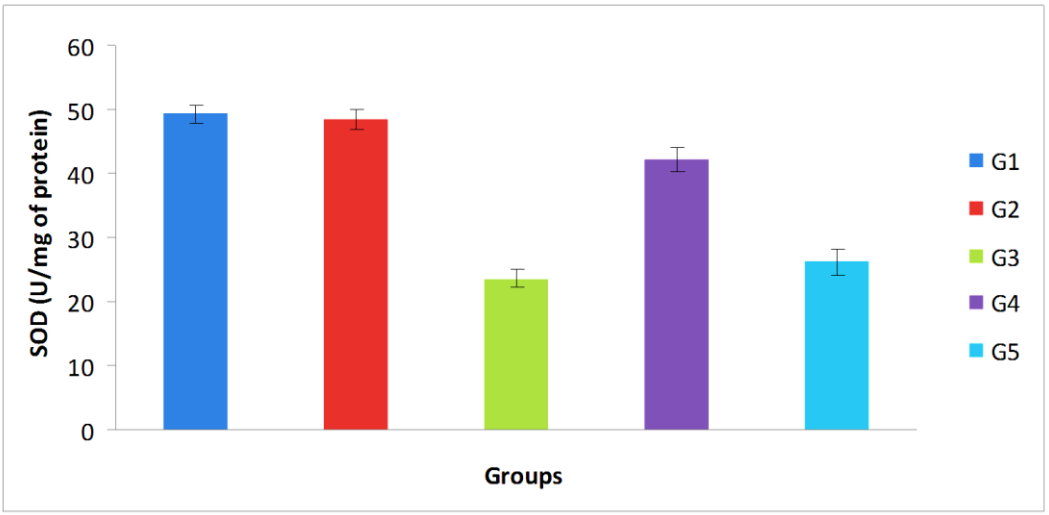


Fig. 17c. Effect of aqueous extract of Acerola berries on liver superoxide dismutase (SOD) activity of paracetamol induced hepatotoxic rats (Mean ± SE, n = 8).



G1 – Control, G2 – Vehicle alone, G3 – Paracetamol alone, G4 – Acerola extract + Paracetamol concomitantly and G5 – Acerola extract prophylactically for 17 days + Paracetamol on day 18 and 19**.**

The liver sections of the rats administered with Acerola extract along with paracetamol (G4) showed normal hepatic architecture on day four of the study (Plate-7). However on 21st day, the liver histology was almost normal, except for mild congestion and focal necrotic areas involving only a very few hepatocytes in certain lobules (Plate-8). In contrast, histopathology of liver from the prophylactic (G5) group of rats, showed central venous congestion diffused and multifocal necrosis of hepatic cells with (Plate-9).

4.4.2 Kidney

Histopathological results of the control (Plate-10) and vehicle administered (Plate-11) animals expressed normal kidney architecture, with rounded glomeruli intimately surrounded by the Bowman's capsule. Paracetamol treated group showed diffused areas of degeneration and necrosis of tubular epithelium and glomeruli on day four (Plate-12). In addition tubular dilatation and haemorrhage was also prominent on day 21 (Plate-13). The kidney section of G4 group of rats on day four (Plate-14) was similar to that of control. However on day 21, tubules showing areas of regeneration was noticed (Plate-15). On the contrary, the prophylactic group (G5) showed cystic dilatation of tubules, diffuse necrosis of the lining cells and glomerulus (Plate-16).

Plate - 10

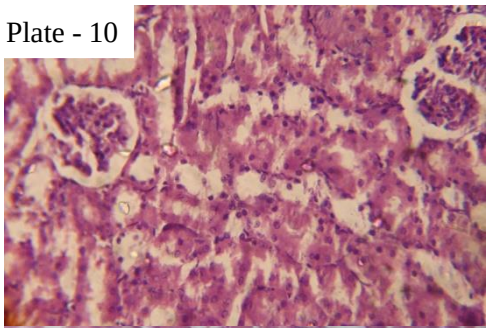


Plate - 4

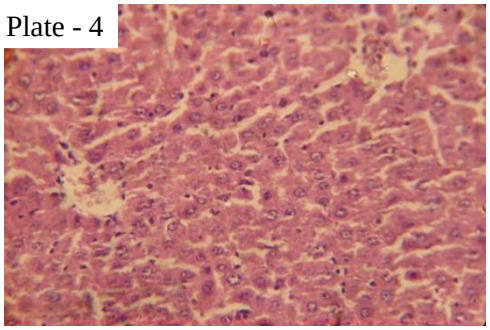


Plate - 5

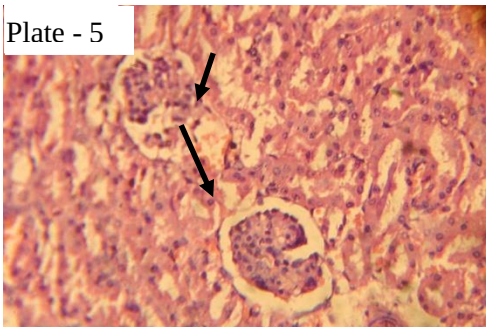


Plate - 6

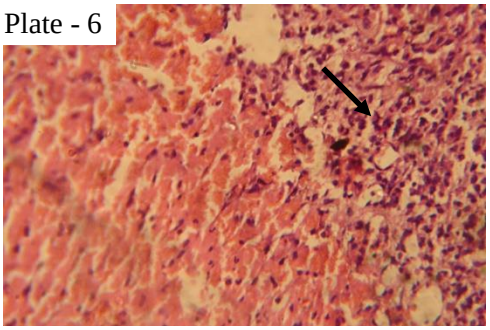


Plate - 7

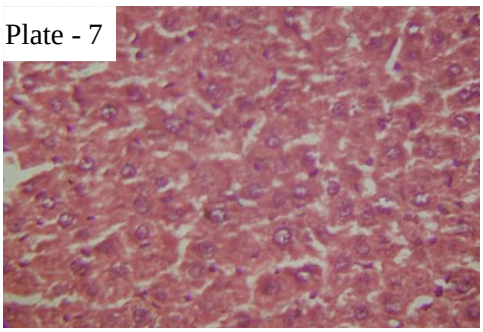


Plate - 8

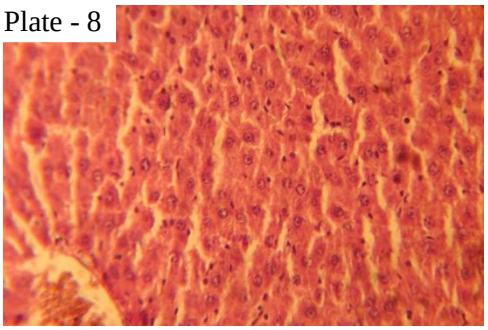


Plate - 9

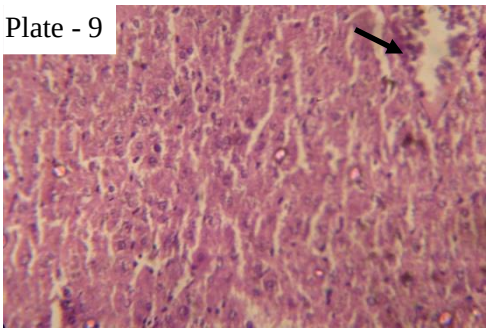


Plate - 3 Control - Day 21 - Liver - showing normal architecture (H&E × 400)

Plate - 4 Vehicle alone - Day 21 - Liver - showing normal architecture (H&E × 400)

Plate - 5 Paracetamol alone - Day four - Liver - showing coagulation necrosis (H&E × 400)

Plate - 6 Paracetamol alone - Day 21 - Liver - showing extensive area of coagulation with inflammatory cell infiltration (H&E × 400)

Plate - 7 Curative group - Day four - Liver - showing normal architecture (H&E × 400)

Plate - 8 Curative group - Day 21 - Liver - showing near normal structure (H&E ×400)

Plate - 9 Protective group - Day 21 - Liver - showing central venal congestion, diffused and multi focal necrosis of hepatocytes (H&E × 400)

Note: Lesions are indicated by arrows

Plate - 11

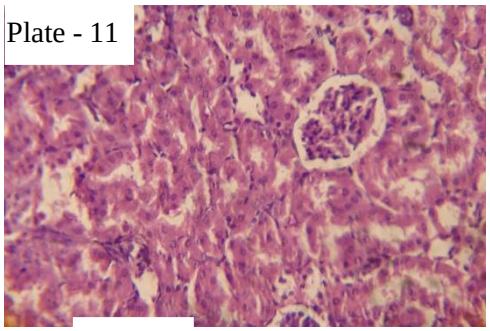


Plate - 13

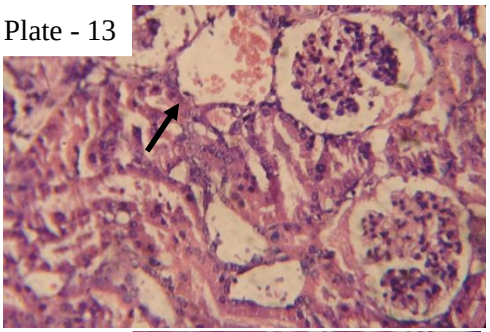


Plate - 14

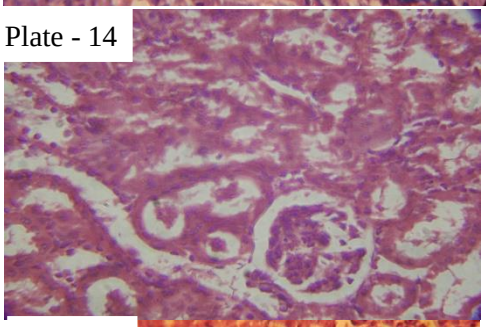


Plate - 15

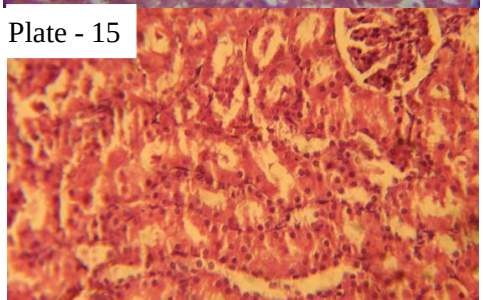


Plate - 16

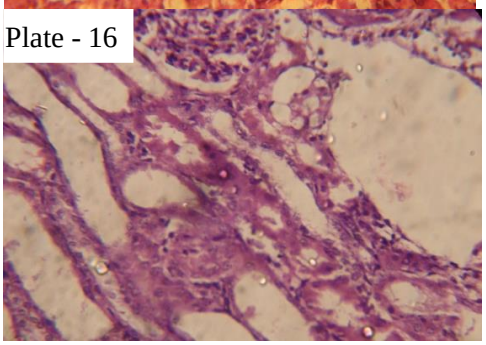


Plate - 14 trol - Day 21 - Kidney - showing normal architecture (H&E × 400)

Plate - 11 Vehicle alone - Day 21 - Kidney - showing normal structure (H&E × 400)

Plate - 12 Paracetamol alone - Day four - Kidney - showing diffused areas of degeneration, necrosis of tubular epithelium and glomeruli (H&E × 400)

Plate - 15 Paracetamol alone - Day 21 - Kidney – showing tubular and glomeruli necrosis, tubular dilatation and haemorrhage (H&E × 400)

Plate - 14 Curative group - Day four - Kidney- showing normal architecture (H&E × 400)

Plate - 16 Curative group - Day 21 - Kidney - tubules showing areas of regeneration (H&E × 400)

Plate – 16 Protective group - Day 21 - Kidney- showing cystic dilatation of tubules, diffuse necrosis of lining cells and glomerular necrosis (H&E × 400)

Note: Lesions are indicated by arrows

Discussion

5. DISCUSSION

Oxidative stress is a harmful condition characterized by excess generation of reactive oxygen species (ROS) which adversely affect the health and productivity of the animal. Overdosing with paracetamol could serve as an animal model for toxic drug interaction in liver due to oxidative stress, which might lead to hepatotoxicity brought about by the metabolites of paracetamol. Antioxidant molecules protect cells against ROS by scavenging them or by promoting their decomposition and therefore play a vital role in health promoting activities. There is increasing interest in the use of naturally occurring antioxidants since synthetic antioxidants require broad and expensive tests to determine their food safety (Frankel *et al.,* 1995). *Malphigia glabra* (Acerola) is well known to be one of the best natural sources of antioxidants such as vitamin C, carotenoids and polyphenols.

5.1 BODY WEIGHT

Increased catabolism of proteins would lead to muscle wasting and reduction in the body weight (Vetrichelvan *et al*., 2002). Absence of significant reduction in the body weight following paracetamol administration might be due to the utilization of other body reserves such as fat for energy purpose.

In the study, all the five groups of rats showed a gradual increase in weight gain. No marked variation was observed in the body weight of rats between groups G2, G3, G4 and G5 throughout the entire experiment. However, on 21st day of the experiment, a significant ($P \leq 0.05$) increase in body weight was observed in the control (G1) rats, when compared to all other groups. On the other hand, in paracetamol administered (G3) group, reduced ($P > 0.05$) body weight was noticed when compared to other groups of rats.

Kumar *et al.* (2001) reported that hepatotoxicity induced with paracetamol (750 mg/kg b.w., p.o.) in Wistar rats, decreased the body weight significantly, which was effectively normalized by administering leaf extract of *Tridax Procumbens,* which contains antioxidants such as polyphenols. Abuelgasim *et al.* (2008) reported reduction in body weight of rats treated with CCl_4 and this was significantly improved in rats treated with seed extract of *Lepidium sativum* administration. They opined that loss of body weight might be due anorexia produced by CCl_4 induced hepatotoxicity, which was cured by the use of *Lepidium sativum*. In the current study, the body weight reduction in G3 animals was improved in G4 group of rats, which might be attributed to the antioxidant property of *Malphigia glabra*.

5.2 HAEMATOLOGICAL PARAMETERS

5.2.1 Total Erythrocyte Count (TEC) and Haemoglobin (Hb) Concentration

The inability of the damaged hepatic parenchyma to synthesize erythropoietinogen, reduced feed intake, absorption and metabolism of nutrients leads to reduction in TEC and Hb concentration. In the present study, TEC and Hb concentration was decreased in paracetamol administered rats on the later stage of the experiment (on day 10 and 21). This is in accordance with the study of Bhaumik and Sharma (2002), who reported a significant decrease in Hb concentration and TEC in rabbits induced with single intravenous injection of paracetamol @ 400 mg/kg b.w.

The vehicle administered (G2) group of rats showed a reduction in Hb concentration during the final stage of experiment, when compared to control (G1) rats. Earlier studies conducted by Lambert (2003) proved that administration of a high sucrose diet in rats resulted in hepatic damage due to impaired mitochondrial function. In the current experiment, reduced Hb noticed in G2 rats

might be due to the hepatic damage produced by the sugar. The increased serum ALT activity observed in G2 group also supported the above finding.

The G4 group of rats in which both paracetamol and the berry extract were given simultaneously, showed a near normal value of TEC and Hb concentration indicating the hepatocurative effect of the extract which might have improved the erthropoietic activity of the hepatic cells. This is in accordance with the results of Abuelgasim *et al.* (2008) who reported reduction of TEC and Hb concentration in rats treated with CCl_4, which was significantly normalized by administration of seed extract of *Lepidium sativum* due to its antioxidant property. The prophylactic (G5) group of rats did not show any significant changes in TEC and Hb concentration throughout the entire study.

5.2.2 Total Leukocyte Count (TLC)

Neutrophils are the first line of defence during acute inflammation, which are later replaced by monocytes that get transformed to macrophages. An increase in the TLC (leukocytosis) might be due to intoxications, infections and tissue necrosis (Benjamin, 2005). This might be as a result of participation of neutrophils and monocytes in the process of phagocytosis as scavengers for a wide variety of particulate material.

A significantly ($P \leq 0.05$) elevated TLC was observed in the paracetamol administered (G3) group, when compared to all other groups of rats, which was in agreement with the finding of Kumar *et al.* (2001), who reported leukocytosis in Wistar rats on paracetamol administration (750mg/kg b.w.). Bhaumik and Sharma (2002) induced hepatic damage in rabbits by single intravenous injection of paracetamol @ 400 mg/kg b.w. and observed significant increase in TLC. Leukocytosis observed in this study might be due to hepatotoxicity produced by paracetamol.

The G4 group of rats did not show any significant (P> 0.05) variation in the TLC value when compared to the control (G1) group. This suggested the curative effect of the berry extract on paracetamol induced cytotoxicity in rats. The high concentration of vitamin C (1100-1400 mg/100g) and polyphenols (3300-4400 mg/100g) present in the extract would have protected the hepatocytes from oxidative damage. The result is in agreement with Kumar *et al.* (2001) who induced hepatotoxicity in Wistar rats with paracetamol (750 mg/kg b.w., p.o.) and reported significantly increased TLC, which was effectively brought down by administering leaf extract of *Tridax Procumbens*, a plant rich in antioxidants.

In the prophylactic study, a significantly (P≤ 0.05) increased TLC value was observed in the G5 group after paracetamol administration (day 21). This showed that the components of Acerola berry that were responsible for the cytoprotective effect could not get accumulated in sufficient quantity in the body to exert a sustained protective effect after paracetamol administration.

5.2.3 Differential Leukocyte Count (DLC)

In the present study, monocytosis was prominent in paracetamol alone administered (G3) group of rats subjected to paracetamol toxicity. Benjamin (2005) reported that monocytes are involved in the process of phagocytosis as scavengers for a wide variety of particulate materials. Liu *et al.* (2004) stated that in paracetamol induced hepatopathy, non parenchymal cells such as kupffer cells, natural killer cells, and neutrophils play a role during the generation of reactive oxygen species. So, monocytosis observed in G3 group might be indicative of hepatic damage.

Treatment with the berry extract, along with paracetamol in G4 group of rats resulted in normal values of DLC, indicated the curative effect of the extract. The hepatoprotective group (G5) showed a significant (P≤ 0.05) increase in the monocyte count, which might reflect the participation of monocytes in the process

of phagocytosis. Therefore the result suggested that pre-treatment of rats with the berry extract did not possess any protective effect on subsequent paracetamol administration.

5.3 BIOCHEMICAL PARAMETERS

5.3.1 Serum Enzymes

5.3.1.1 Alanine Amino Transferase (ALT)

Alanine amino transferase is considered to be liver specific and is present at higher concentration in the cytoplasm of hepatocytes. The enzyme catalyses the conversion of alanine to pyruvate and glutamate.

L-Alanine + 2-Oxoglutarate ⟷ Pyruvate + L-Glutamate

Serum ALT activity increases with hepatocellular damage or necrosis and hepatic cell degeneration due to altered membrane permeability. The administration of paracetamol resulted in elevated activities of liver enzymes in serum, an indicator of cellular leakage and loss of activities of cell membranes in liver. The elevation of liver enzymes, especially ALT has more importance as a specific marker of liver injury due to toxic drugs, alcohol and virus.

In the present study, a significant ($P \leq 0.05$) increase was noticed in the ALT activity of paracetamol administered (G3) group of rats when compared to all other groups. This is in agreement with earlier findings of Chattopadhyay *et al*. (1992), Bhanwra *et al.* (2000) whose reports showed a marked elevation of serum ALT activity within 24 to 48 hrs after administration of paracetamol p.o. @ 2 g/kg b.w. Elevated serum ALT activity is positively correlated with the number of damaged hepatocytes. Therefore an elevated activity of serum ALT in G3 rats was indicative of hepatic damage.

The significantly (P≤ 0.05) reduced serum ALT activity observed in curative group of rats (G4), treated with berry extract along with paracetamol as compared to those treated with paracetamol alone indicated that the berry extract had reduced the hepatic damage. This result is supported by the earlier findings of Chattopadhyay *et al.* (1992) and Antony *et al*. (2006), who observed a significantly reduced serum ALT activity in hepatotoxic rats, on administration of plant extracts (such as *Azadirachta indica* and *Centella asiatica* respectively) which were rich in antioxidants.

The ALT activity of G4 rats was higher than control (G1) rats, but was similar to that of vehicle administered (G2) rats. This increase might be due to the effect of sugar which was used as vehicle. Earlier studies conducted by Lambert (2003) also proved that administration of a high sucrose diet in rats resulted in hepatic damage due to impaired mitochondrial function.

The G5 group of rats, fed with Acerola extract for initial 17 days and then induced hepatotoxicity with paracetamol on day 18 and 19 of the experiment showed significantly (P≤ 0.05) elevated serum ALT activity on 21st day of the experiment. This revealed that the berry extract did not possess any prophylactic effect and so there was no reduction in hepatic damage caused by paracetamol. Since vitamin C and polyphenols in the extract are water soluble, they might have eliminated rapidly from the body and hence could not have extended any protective effect.

5.3.1.2 Aspartate Amino Transferase (AST)

Aspartate amino transferase is found in all body cells and it plays a vital role in the conversion of amino acids to keto acids (Sallie *et al*., 1999).

L-Aspartate + 2-Oxoglutarate ⟷ Oxaloacetate + L-Glutamate

Necrosis or membrane damage of cells releases the enzyme into the circulation. So, increased AST chiefly indicates muscle and liver damage. It is leaked out both from cytoplasm and mitochondria of hepatocytes and other cells. An elevated activity of serum AST is always noticed along with increased activity of serum ALT.

In the present study, paracetamol administration resulted in significantly ($P \leq 0.05$) elevated activity of serum AST, which might be due to the release of this enzyme from the disrupted cells. Chattopadhyay *et al*. (1992), Bhanwra *et al.* (2000), Asha (2001) also observed elevated activity of serum AST in rats administered with paracetamol @ 2 g/kg b.w.

The G4 group of rats had an AST activity similar to control G1 rats. This indicated that the aqueous extract of the berry had curative effect against paracetamol induced cell damage. The result was similar to that obtained by Olalaye *et al.* (2006) with *Alchornea cordifolia* leaf extract in paracetamol induced hepatotoxic adult Wistar albino rats.

On the contrary, the prophylactic (G5) group of rats showed a significantly ($P \leq 0.05$) increased serum AST activity after paracetamol administration, which indicated that the berry extract might not possess any hepatoprotective effect. This may be due to the less half life of the prime components of Acerola such as vitamin C and polyphenols.

5.3.1.3 Alkaline Phosphatase (ALP)

Alkaline phosphatase is present in nearly all tissues and constitutes a group of enzymes that are involved in the hydrolysis of phosphate monoesters at an alkaline pH. Elevated levels of ALP suggests conditions such liver damage, bone resorption, renal disease, gastrointestinal lesions and haemolysis.

In this study, the ALP activity in G3 group of rats was significantly ($P \leq 0.05$) higher when compared to all other groups of rats. This along with a significant increase in ALT and AST was indicative of oxidative hepatic damage. This is in agreement with the findings of earlier workers Chattopadhyay *et al*. (1992), Bhanwra *et al.* (2000), Asha (2001), Olaleye *et al.* (2006) and Ojo *et al*. (2006) whose reports showed a marked elevation of serum ALT, AST and ALP levels within 24 to 48 h on administration of paracetamol (2 g/kg b.w. p.o.), signifying liver damage.

The G4 group of rats had a significantly ($P \leq 0.05$) reduced ALP activity when compared to the paracetamol administered (G3) group which revealed the efficiency of the Acerola berry extract to cure the paracetamol induced cell damage. The result correlates with the finding of Asha (2001), who observed a decreased serum ALP activity on administration of tender leaves of *Marmodica subangulata* to paracetamol induced hepatotoxic rats.

In G5 group, the ALP activity was significantly ($P \leq 0.05$) high on day 21 subsequent to paracetamol administration. From the observation it appears that Acerola berry extract could not offer any prophylactic effect against the damage of cells caused by paracetamol. This is again supported by the increased levels of serum AST and ALT in this group.

5.3.2 Serum Protein Profile and A:G Ratio

Serum protein is composed of albumin and globulin. Early phase of hepatic damage does not alter the synthesis of serum albumin. However, during severe liver injury, the albumin fraction would be diminished and the globulin fraction get increased, leading to low A:G ratio(Benjamin, 2005).

In the present study, the total protein, albumin, globulin and A:G ratio observed in different groups of rats did not differ significantly. Estimation of total

protein, albumin, globulin and A:G ratio is usually of little value in the assessment of liver function or disease (Benjamin, 2005). It has been reported that a marked reduction in hepatic function usually to less than 20 % of normal is necessary to induce hypoalbuminemia due to decreased synthesis (Evans, 1998). This indicated that paracetamol induced liver damage in the current experiment might not be more than 80 % to alter the serum levels of these biochemical parameters as reported by Evans (1998). However, a relatively decreased (insignificant) level of serum total protein and albumin and a lower A:G ratio was recorded in paracetamol induced hepatotoxic (G3) group of rats which may be an indication of hepatic damage.

5.3.3 Serum Total Cholesterol

To certain extent, the liver function can be assessed by the serum total cholesterol concentration. Cholesterol is synthesised in almost all cells, especially liver. The liver also functions to excrete cholesterol through the bile. Damage to hepatocytes and renal diseases may result in elevated serum cholesterol levels (Benjamin, 2005).

In the current study, paracetamol administered (G3) group had a significantly ($P \leq 0.05$) elevated serum total cholesterol concentration when compared to all other groups of rats. This gives a sign of liver and kidney damage. Ojo *et al.* (2006) reported that paracetamol (2g/kg b.w., p.o.) produced oxidative liver damage which was indicated by elevated serum cholesterol level. They suggested that administration of rats with paracetamol altered the cell membrane structure and function leading to cell damage resulting in high serum cholesterol concentration. In the present experiment, the elevated serum cholesterol concentration observed might be due to the altered cell membrane structure and function leading to cell damage as suggested by Ojo *et al.* (2006).

In the G4 group of rats, the serum cholesterol concentration was significantly (P≤ 0.05) lower than paracetamol administered (G3) group. The result suggested that co- administration of berry extract and paracetamol prevented the alteration of cell lipid membrane and hence prevented the increase in the level of serum cholesterol. The results obtained are also in accordance with the study conducted by Ojo *et al.* (2006) in which, administration of paracetamol induced hepatotoxic rats with green tea and lemon grass extracts (rich in polyphenols) prevented the alteration in the level of serum cholesterol.

However, an elevated level of serum cholesterol was observed in both the curative (G4) and the vehicle administered (G2) groups of rats when compared to the control group. This increase may be due to the effect of sugar which was used as vehicle for the administration of paracetamol. Studies conducted by Lambert (2003) also proved that administration of a high sucrose diet in rats resulted in hepatic damage due to impaired mitochondrial function.

The hepatoprotective (G5) group, showed a significantly (P≤ 0.05) increased serum total cholesterol concentration after paracetamol administration, which suggested that the berry extract did not possess any prophylactic effect.

5.3.4 Blood Urea Nitrogen (BUN)

The measurement of urea in serum is mainly used to assess kidney function. Urea is the major nitrogen containing metabolite which is produced in the liver by the catabolism of protein. It accounts for more than 75% of the non protein nitrogen substances eventually excreted. Therefore increase in BUN level is indicative of both kidney and liver damage (Benjamin, 2005).

In the current experiment, significantly (P≤ 0.05) elevated BUN level was observed in paracetamol induced hepatotoxic (G3) group, which might indicate hepatorenal damage as reported by El-Ridi *et al.* (2000). The rats in G4 group did

not show any significant (P> 0.05) variation in BUN level when compared to the control group. This result suggested the curative effect of the berry extract on paracetamol induced hepatorenal toxicity in rats. Satyanarayana *et al.* (2001) noticed that administration of quercetin (a bioflavonoid with antioxidant potential) @ 2 mg/kg b.w. markedly brought down the elevated BUN level indicating its curative effect on cyclosporine induced oxidative stress and nephrotoxicity in rats. In the present study the antioxidants in Acerola extract might have protected the cells against the hepatorenal damage.

On the contrary, the G5 rats fed with Acerola extract for initial 17 days and then administered with paracetamol on day 18 and 19 of the experiment showed significantly (P≤ 0.05) elevated levels of BUN on the final day (21st day) of the experiment. This indicated that Acerola berry extract could not offer considerable prophylactic and sustained activity against the oxidative hepatorenal damage caused by paracetamol administration.

5.3.5 Serum Total, Direct and Indirect Bilirubin

Bilirubin is produced by enzymatic degradation of haemoglobin liberated from senescent erythrocytes during their destruction. It is then secreted in the bile which imparts yellow colour to plasma. Estimation of bilirubin levels in serum helps to determine the functioning of liver. Direct bilirubin is that which has been taken up by the liver cells and get conjugated to form the water soluble bilirubin, diglucuronide. Indirect bilirubin is that which has not been conjugated with glucuronic acid. Elevated levels of serum total, direct and indirect bilirubin was observed during toxic hepatocellular damage (Benjamin, 2005).

In the present study, significantly (P≤ 0.05) high bilirubin level was noticed in the paracetamol administered group (G3). This result is in agreement with the report of Roy *et al*. (2006) who observed an increase in bilirubin level in rats on paracetamol administration p.o. @ 1g/kg b.w. in three divided doses.

Bilirubin concentration was restored to normal level in G4 rats treated with the berry extract. So it could be inferred that the berry extract can cure the damaging effect of paracetamol in hepatocytes. Similar result was obtained by Bose *et al*. (2007) with methanol extract of the plant *Eupatorium ayapana* in CCl_4 induced hepatotoxicity in rats.

In the protective study, the G5 group showed significantly ($P \leq 0.05$) elevated levels of serum total, direct and indirect bilirubin on the final day of the experiment. The result revealed that the berry extract did not possess any prophylactic effect and so there was no reduction in hepatic damage even after administration of the extract for 17 days before paracetamol administration.

5.3.6 Antioxidant Profile

5.3.6.1 Serum and Liver Reduced Glutathione

Hepatorenal toxicity caused by acetaminophen is mediated by a reactive metabolite which is normally detoxified by cellular reserves of reduced glutathione (Flanagan and Meredith, 1991). Glutathione (GSH) is a powerful scavenger of free radicals and it plays a major role in determining detoxifying capacity of the organism. However, during this process the GSH will get oxidized resulting in further depletion of GSH stores. Therefore, GSH is important to maintain cellular macromolecules in functional state and it serves as a key determinant to assess the extent of paracetamol induced hepatic damage (Kuralay *et al.,* 1998; Yanpallewar *et al.,* 2002).

A significant decrease in the level of intracellular GSH may lead to oxidative stress (Moore *et al.*, 1985, Corcoran and Wong, 1986). In the current study, a significantly ($P \leq 0.05$) low serum and liver GSH noticed in G3 rats is indicative of oxidative liver damage. This is in agreement with the result of

Muriel *et al.* (1993) who reported a significantly low GSH content in Wistar male rats suggesting liver damage, after paracetamol administration.

The G4 group of rats, supplemented with Acerola along with paracetamol showed a significantly ($P \leq 0.05$) elevated level of serum and liver reduced glutathione when compared to paracetamol (G3) administered group of rats. This revealed that the berry extract possessed hepatocurative effect and as a result there was reduction in hepatic damage caused by paracetamol. The result was similar to that obtained by El-Ridi *et al.* (2000) with vitamin C in paracetamol induced hepatorenal toxicity in male rats. Olalaye *et al.* (2006) also observed a markedly increased GSH content when *Alchornea cordifolia* leaf extract rich in flavanoids, was administered to paracetamol (2 g/kg b.w., p.o.) induced hepatotoxic adult Wistar albino rats.

In the protective study, a significantly ($P \leq 0.05$) low serum and liver glutathione activity was observed in the (G5) group, after paracetamol administration. So it can be inferred that the berry extract could not yield any protective effect against the hepatic damage caused by paracetamol in rats. This might be due to the less half life of vitamin C and polyphenols, as discussed earlier.

5.3.6.2 Lipid Peroxides in Liver

Lipid peroxidation is an autocatalytic process which is a common consequence of cell death. In case of paracetamol toxicity, lipid peroxidation resulting from oxidative stress was responsible for the initiation and progress of liver damage (Albano *et al.*, 1985). It is a molecular mechanism of cell injury, during which oxidation of poly unsaturated lipids takes place leading to the generation of peroxide and lipid hydro peroxides. These can further decompose to yield a wide range of cytotoxic products, which are thio barbituric acid reactive substances (TBARS), such as malondialdehyde (MDA). Under physiological

conditions, low concentrations of lipid peroxides are found in tissues and cells. However during oxidative stress more lipid peroxides are formed and released in to the serum due to cell damage.

In the present study, a significant ($P \leq 0.05$) increase in the level of lipid peroxides was observed in the liver tissue of paracetamol administered (G3) group of rats (659.14 ± 47.31 nM/g) when compared to the control (G1) group (355.25 ± 14.78 nM/g). Singh *et al.* (2006) also reported a significant increase in the formation of MDA (end products of lipid peroxidation) and considered it as an indication of liver toxicity. Dash *et al*. (2007) reported that, paracetamol @ 750 mg/kg b.w. in rats resulted in a significant elevation in levels of serum TBARS and decrease in GSH activity, suggestive of hepatic damage. Hence the significantly high level of lipid peroxides observed in G3 group can be attributed to the hepatocellular damage.

The significantly ($P \leq 0.05$) reduced lipid peroxidation level (401.50 ± 22.14 nM/g) observed in hepatocurative group of rats (G4), indicated that the berry extract had ameliorated the hepatic damage caused by paracetamol. The effect may be due to the antioxidant activity of the extract to scavenge the free radicals. This result is supported by the earlier findings of Ojo *et al.* (2006) who showed inhibitive effects of green tea and lemon grass extracts on paracetamol induced lipid peroxidation in rats, which are attributed to the antioxidant property of the extracts.

A significant ($P \leq 0.05$) increase in the level of liver lipid peroxides was observed in the hepatoprotective (G5) group (850.29 ± 83.98 nM/g), after administration of paracetamol, which suggested that the berry extract did not possess any hepatoprotective effect. This is also supported by increased level of serum AST and ALT and a decreased level of reduced glutathione in G5 rats.

5.3.6.2 Superoxide Dismutase (SOD)

Body cells have a number of mechanisms to protect themselves from the toxic effects of free radicals generated by various drugs. Superoxide dismutase removes superoxides by converting it into hydrogen peroxide that could be rapidly converted into water by catalase and glutathione peroxidase (Gupta *et al.,* 2004). Superoxide dismutase has been reported as one of the most important antioxidant enzyme. It scavenges the superoxide anions to form hydrogen peroxide and diminishing the toxic effect caused by this radical (Curtis *et al*. 1972). These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage.

In the current study, significantly ($P \leq 0.05$) decreased values of liver SOD observed in paracetamol induced hepatotoxic (G3) group might be due to the enzyme inactivating activity of the generated reactive oxygen species. Similar result was reported in the studies conducted by Dash *et al*. (2007) who found that paracetamol @ 750 mg/kg b.w. caused significantly decreased activities of SOD. Chattopadhyay and Bandyopadhyay (2005) also observed a significant reduction of liver superoxide dismutase (SOD) activity representing hepatic damage, in rats administered with paracetamol p.o. @ 2 g/kg b.w.

In the curative study, the G4 group of rats, supplemented with Acerola along with paracetamol showed a significantly ($P \leq 0.05$) elevated level of liver SOD when compared to paracetamol administered (G3) group of rats. This indicated that the berry extract possessed hepatocurative effect which might be due to its antioxidant property. The result was similar to that reported by Bose *et al*. (2007) with extract of *Eupatorium ayapana* in CCl_4 induced hepatotoxicity in rats and Gupta *et al*. (2004) with methanolic extract of the plant *Bauhinia racemosa* on both paracetamol and CCl_4 induced hepatotoxic rats.

In the prophylactic study, a significantly (P≤ 0.05) reduced liver SOD activity was observed in the G5 group, after paracetamol administration. So it could be inferred that the berry extract could not possess any protective effect against the hepatic damage caused by paracetamol in rats.

5.4 HISTOPATHOLOGICAL EXAMINATION

5.4.1 Liver

Histopathological examination of liver in control group of rats showed normal architecture of portal triad, and central vein with radiating hepatocytes.

The paracetamol administered (G3) group showed massive coagulation necrosis in the hepatic lobules. On day four of the experiment, the lesions were less severe when compared to day 21. Various degrees of pathological changes starting from cloudy swelling, necrosis of hepatic cells and central lobular fatty degeneration were noticed on day 21. There were extensive areas of sinusoidal congestion, multifocal diffuse haemorrhage, confluent centrilobular necrosis, cell inflammation, eosinophilic cytoplasm and infiltration of inflammatory cells. Hepatic necrosis was found to be greater in zone 3, where drug metabolising enzymes were found in highest concentration and the oxygen tension was lowest in the sinusoidal blood and these indicated hepatocellular degeneration.

The above result was comparable with the findings of Hewawasam *et al*. (2003) who reported confluent necrosis with vacoulation in hepatocytes after paracetamol administration. Dixon *et al*. (1971) observed necrosis of liver parenchyma, marked congestion and disrupted sinusoids packed with red blood cells, 48 h after paracetamol (2.5 g/kg b.w., p.o.) administration. Moreover, Mathew (2005) reported that paracetamol @ 3 g/kg b.w. for three days resulted in centrilobular coagulative necrosis of the liver due to covalent binding of the

oxidation products of paracetamol, to the sulfhydryl groups of the protein resulting in the cell damage and thereby necrosis of the liver.

On day four and 21 of the study, in the hepatocurative (G4) group, the histological changes induced by paracetamol administration was reversed by co-administration of Acerola extract. Focal necrotic areas involving a few hepatocytes were observed in certain lobules. It might be due to the free radical scavenging effect of the phenolic components present in the extract that may prevent the toxic chemical reactions that generate ROS thereby producing lipid peroxidation and molecular changes, which ultimately leads to liver tissue necrosis. Moresco *et al.* (2007) opined that the scavenging of free radicals by the *Syzygium cumini* may have provided protection against the hepatotoxicity.

Histopathology of liver from the prophylactic (G5) group of rats showed lesions similar to that observed in paracetamol administered (G3) rats. This suggested that pretreatment of rats with the extract could not prevent paracetamol induced damage in the hepatic structure and did not protect the liver tissue from necrotic and fatty degenerative changes.

5.4.2 Kidney

Histologically, the kidney sections of animals in G3 group administered with paracetamol had tubular dilatation on day four. Tubular and glomerular necrosis and glomerular haemorrhages were observed on day 21 of the experiment. Co-administration of the extract in G4 group of rats ameliorated the toxic manifestations in the kidney on both day four and 21. The result revealed marked curative effect of Acerola extract on paracetamol induced liver damage in rats. This indicated that Acerola possesses antioxidant properties which might contribute to its hepatocurative effect. Palani *et al.* (2009) reported that paracetamol @ 750 mg/kg b.w. produced severe tubular necrosis and degeneration and the rats treated with ethanolic extract of *Pimpinella tirupatiensis*

(500mg/kg b.w.), a potent antioxidant showed normal tubular pattern with a mild degree of swelling, necrosis and degranulation.

However in the protective study, the G5 rats showed lesions similar to that observed in paracetamol administered rats, which suggested that Acerola berry extract do not possess any prophylactic effect.

5.5 CONCLUSION

The present study on antioxidant potential of *Malphigia glabra* (Acerola) berries in rats revealed that the berry extract @ 20 ml/kg b.w., p.o. could cure hepatic damage and associated haematological and biochemical alterations in adult rats with paracetamol induced hepatotoxicity. The study also revealed that the active antioxidants of Acerola could not be stored in the body (may be due to its water soluble nature) to produce a prophylactic effect against paracetamol toxicity. However, further studies using some more models of experimental hepatic damage are required to elucidate exact molecular and biochemical mechanisms involved and to establish its role as a hepatocurative agent.

Summary

6. SUMMARY

The current study was performed to evaluate the hepatocurative and hepatoprotective effects of aqueous extract of mature fruits of *Malphigia glabra* (Acerola) berries on paracetamol induced hepatotoxic rats.

Experiment was carried out in 42 adult male Wistar albino rats weighing 150 – 200 g, which were randomly divided into five groups. The groups G1, G2 and G5 comprised of eight animals each and groups G3 and G4 consisted of nine animals each. Rats in G1 represented the control group. The G2 served as vehicle alone administered group, in which the rats were given 40 % sucrose syrup p.o. @10 ml/kg b.w. on day one and two and then at three days interval for 21 days. Group G3 constituted the untreated paracetamol administered rats, in which toxicity was induced with paracetamol @ 2 g/kg b.w. p.o., on day one and two and at every three days interval upto day 18. The G4 rats formed the curative group which were administered with paracetamol @ 2 g/kg b.w. p.o., on day one and two and at every three days interval upto day 18 along with Acerola berry extract @ 20 ml/ kg b.w. p.o., for 21 days. Rats in G5 formed the protective group which were administered with Acerola berry extract @ 20 ml/ kg b.w. p.o. for 17 days and then induced hepatotoxicity with paracetamol @ 2 g/kg b.w. p.o., on day 18 and 19.

Body weight was recorded at weekly intervals. Blood samples were collected from eight animals in each group on day zero, four, 10, and 21. An additional blood collection was carried out in G5 group of rats, on day 18 of the experiment. The blood samples were subjected to various haematological and serum biochemical analysis. Haematological parameters included the total erythrocyte count (TEC), haemoglobin (Hb) concentration, total leukocyte count (TLC) and differential leukocyte count (DLC). The serum biochemical parameters examined were alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total cholesterol, total protein, albumin,

globulin, albumin:globulin (A:G), blood urea nitrogen (BUN), total, direct and indirect bilirubin as well as reduced glutathione. One animal each from G3 and G4 was euthanized on day four and rest of the rats were euthanized on day 21. Levels of liver reduced glutathione, lipid peroxides and superoxide dismutase (SOD) on 21st day of the experiment were estimated. Representative samples of liver and kidney tissues collected on day four and 21 were subjected to histopathological examination.

Total erythrocyte count and Hb concentration were decreased in paracetamol induced hepatotoxic rats on the latter stage (day 10 and 21) of the experiment, while the G4 group of rats recorded a near normal TEC and Hb value. The G5 group of rats did not show any significant changes in TEC and Hb concentration. Significantly elevated TLC was observed in G3 group on day 10 and 21, when compared to all other groups of rats. The G4 group of rats did not show any significant variation in the TLC value when compared to the control (G1) group. In the hepatoprotective (G5) group, a significantly increased TLC value was observed after paracetamol administration (on day 21).

Monocytosis was prominent in G3 group of rats subjected to hepatopathy. Treatment with the berry extract, along with paracetamol (G4) resulted in normal values of DLC, which might indicate curative effect of the extract. Conversely, the hepatoprotective group (G5) showed significant increase in the monocyte count.

Paracetamol administration (2 g/kg b.w.) resulted in significantly elevated activity of serum enzyme markers, ALT, AST and ALP when compared to all other groups. The hepatocurative (G4) group of rats had significantly reduced serum ALT, AST and ALP activities. The serum ALT and ALP activities of G4 rats were higher than the control (G1) rats, but was similar to that of vehicle administered (G2) rats. On the contrary, the hepatoprotective group (G5) showed

a significantly increased serum ALT, AST and ALP activities after paracetamol administration on day 21.

Administration of paracetamol resulted in a significantly elevated serum level of total cholesterol, BUN, as well as total, direct and indirect bilirubin. In G4 group of rats, these values were effectively restored. However, an elevated serum total cholesterol concentration was observed in both the hepatocurative (G4) and the vehicle administered (G2) group of rats when compared to the control group (G1). The hepatoprotective group (G5), showed significantly increased serum total cholesterol, BUN, total, direct and indirect bilirubin concentrations on paracetamol administration.

There was no substantial change in the mean body weight, serum total protein, albumin, globulin and A:G ratio in different groups of rats throughout the entire experiment. However in G3 rats with paracetamol induced hepatotoxicity, the mean body weight, serum total protein, albumin, globulin and A:G ratio were less (insignificant).

Paracetamol administration significantly decreased the serum and liver reduced glutathione content and liver SOD activity and this was successfully normalised in G4 group of rats, supplemented with Acerola along with paracetamol. But in the protective study, a significantly low serum and liver glutathione content and liver SOD activity was observed in the (G5) group, after administration of paracetamol.

A significantly increased level of lipid peroxides was observed in the liver tissue of G3 group (administered with paracetamol alone) and G5 group (hepatoprotective) after paracetamol administration. However, in the curative study, the G4 group of rats showed a significantly decreased level of liver lipid peroxides when compared to G3 group of rats.

Histopathology of G3 rats revealed necrosis of hepatic cells, diffused haemorrhage and central venous congestion in liver and tubular dilatation with congestion in kidney. The lesions observed in G3 (rats administered with paracetamol alone) were effectively prevented in G4 rats which were given acerola extract in addition to paracetamol.

Based on the findings of the present study on the antioxidant potential of *Malphigia glabra* (Acerola) berries in rats, it could be summed up as follows:

1. The berry extract possessed significant hepatocurative and antioxidant effect.

2. Aqueous berry extract did not show any prophylactic effect on oxidative hepatic damage.

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ANTIOXIDANT POTENTIAL OF *Malphigia glabra* (ACEROLA) BERRIES IN RATS

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Abstract of the thesis
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ABSTRACT

The present study was designed to assess the effect of aqueous extract of mature fruits of *Malphigia glabra* (Acerola) berries on paracetamol induced hepatotoxicity in rats.

Forty-two adult male Wistar albino rats were used for the experiment. The rats were randomly divided into five groups with eight animals each in G1, G2 and G5 and nine animals in G3 and G4. Group G1 served as normal control rats. The G2 (untreated vehicle alone) rats were administered with 40 % sucrose syrup p.o. @ 10 ml/kg b.w. on day one and two and then at three days interval for 21 days. The G3 group of rats were administered with paracetamol @ 2 g/kg b.w. p.o., on day one and two and at every three days interval upto day 18. The G4 (curative group) rats were administered with paracetamol p.o. @ 2 g/kg b.w. on day one and two and at every three days interval upto day 18 and Acerola berry extract (20 ml/ kg b.w., p.o.) for 21 days. The G5 (protective group) rats were given Acerola berry extract p.o. @ 20 ml/ kg b.w. for 17 days and paracetamol p.o. @ 2 g/kg b.w. on day 18 and 19.

Body weight was recorded at weekly intervals. Blood samples were collected from eight animals in each group on day zero, four, 10, and 21. In G5 group, an additional blood collection was made on 18th day of the experiment before administration of paracetamol. The haematological parameters such as total erythrocyte count (TEC), haemoglobin (Hb) concentration, total leukocyte count (TLC) and differential leukocyte count (DLC) and serum biochemical parameters such as alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total cholesterol, total protein, albumin, globulin, albumin:globulin, blood urea nitrogen (BUN), total bilirubin, direct bilirubin, indirect bilirubin, reduced glutathione were analysed. One animal each from G3 and G4 was euthanized on day four and rest of the rats were euthanized on day 21. Levels of liver reduced glutathione, lipid peroxides and superoxide dismutase

(SOD) on 21st day were estimated. Representative samples of liver and kidney tissues collected on day four and 21 were subjected to histopathological examination.

Administration of paracetamol in G3 group caused a significant ($P \leq 0.05$) increase in the levels of serum ALT, AST, ALP, total cholesterol, BUN and total, direct and indirect bilirubin while reduced glutathione content was significantly reduced. The activities of liver reduced glutathione and SOD were also decreased significantly whereas the liver lipid peroxide content was significantly increased. Haematological analysis showed significantly decreased TEC and Hb concentration and a significantly increased TLC with monocytosis. No significant ($P > 0.05$) variation was observed in body weight, and in the levels of serum total protein, albumin, globulin and albumin:globulin. Histopathology indicated necrosis of hepatic cells, diffused haemorrhage, central venous congestion and focal coagulation in liver; while tubular dilatation and congestion was observed in kidney.

Administration of Acerola berry extract along with paracetamol in G4 (curative group) rats effectively reversed the levels of serum ALT, AST, ALP, total cholesterol, BUN, total bilirubin (direct and indirect), reduced glutathione (in liver and serum), liver SOD and lipid peroxides in liver to normalcy signifying the antioxidant and hepatocurative effect of the extract. However, the active antioxidant components of *Malphigia glabra* such as vitamin C and polyphenols has a short half life, so that the berry extract could not produce any prophylactic effect against paracetamol induced toxicity in G5 (protective group) rats, evidenced by toxic range of values.