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# EVALUATION OF HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *Eugenia jambolana* (NJAVAL) LEAVES ON PARACETAMOL INDUCED TOXICITY 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 the thesis entitled **"EVALUATION OF HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *Eugenia jambolana* (NJAVAL) LEAVES ON PARACETAMOL INDUCED TOXICITY IN RATS"** is a bonafide record of research work done by me during the course of research and that this 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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## CERTIFICATE

Certified that this thesis, entitled **"EVALUATION OF HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *Eugenia jambolana* (NJAVAL) LEAVES ON PARACETAMOL INDUCED TOXICITY IN RATS"** is a record of research work done independently by **Midhun M.V.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to him.

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# *Introduction*

# 1. INTRODUCTION

Liver is the major and largest visceral organ in the body. It plays an astonishing array of vital functions in the maintenance and performance of the body and has a major role in the metabolism of carbohydrate, protein and fat. Detoxification of potentially toxic chemicals from both inside and outside the body including drugs, alcohol, toxins from intestinal microbes, secretion of bile, production of clotting factors and plasma protein synthesis are the main other important functions. It is roughly triangular in shape and lies below the diaphragm in thoracic region of abdomen. The maintenance of a healthy liver is vital to overall health and well being (Treadway, 1998).

Unfortunately the liver is often abused by environmental toxins, malnutrition, alcohol and over-the-counter drug use, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis and alcoholic liver diseases in human beings. The liver can regenerate itself to some extend. The excessive use of certain drugs like carbon tetra chloride ($CCl_4$), paracetamol, heavy metals and antitubercular drugs like isoniazid and rifampicin can damage the liver. Some other hepatotoxins include mycotoxins like aflatoxin $B_1$ and plants like lantana. Most of the hepatotoxic chemicals damage liver cells mainly by lipid peroxidation and other oxidative damages in the liver.

When the liver cells are damaged the liver marker enzymes like serum alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) are released into the blood and elevated levels of these enzymes are noticed in blood. Elevation of serum transaminase level has invariably been used as an index of liver damage in detecting hepatic necrosis in poisoning with paracetamol (Dixon, 1975). Several biochemical parameters like ALT, AST, ALP and serum bilirubin are used to assess the hepatic function.

Paracetamol (N-acetyl-*p*-amino phenol) also known as acetaminophen is considered to be a safe analgesic and antipyretic drug at therapeutic doses. The high potency and lack of gastrointestinal side effects of this drug have led to the wide spread

use of paracetamol which is regarded as a safer alternative to other NSAIDs for mild to moderate analgesia (Moore *et al.*, 2001). Paracetamol when taken in overdoses results in hepatotoxicity and nephrotoxicity in experimental animals (Kaushal *et al.*,1999). Paracetamol related liver damage occurring with in the recommended dosage is dose dependent especially in case of severe malnutrition (Kurtovic and Riordán, 2003).

Under normal conditions, paracetamol is primarily metabolized in the liver by glucuronidation and sulfation. A proportion of the drug is metabolized by several of the cytochrome P 450 enzymes into a more reactive metabolite, N- acetyl-*p*- benzoquinone imine (NAPQI) which is responsible for the hepatotoxicity. NAPQI is normally detoxified by conjugation with glutathione (GSH) both enzymatically and non-enzymatically to a water soluble harmless product, mercaptopuric acid. In overdose, sulfation and glucuronidation become saturated and GSH is depleted by NAPQI (Makin and Williams, 2000). Excess of NAPQI causes oxidative stress and binds covalently to liver proteins and there by causes the liver damage.

The pharmaceutical imbalance between remedies that protect the liver through the antioxidant properties and drugs that induce hepatotoxicity has prompted and accelerated research on to plants used in folk medicine to treat liver diseases and boost liver functions. Such plants include *Azadirachta indica, Andrographis paniculata, Piper longum, Phyllathus niruri, Curcuma longa, Picrorrhiza kurroa, Camellia sinensis, Silybum marianum and Glycerrhiza glabra* (Iwalokun *et al.*, 2006).

Liver diseases remain as one of the serious health problems. However we do not have satisfactory liver protective drugs in allopathic medicinal practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders, most of which speed up the natural healing process of the liver and they play a key role in the human and animal health. About 80% of the world population relies on the traditional medicine which refers to a broad range of ancient, natural, health care practices including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani which were based on plant materials. Numerous medicinal plants and their formulations were used for liver

disorders in ethno medical practice as well as traditional system of medicine. In India, about 40 polyherbal commercial formulations reported to have hepatoprotective action are being used.

A phytotherapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants. In recent years many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of liver. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available (Gupta *et al.*, 2004). Conventional medicine now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis (Treadway, 1998). In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cells. Scientific evaluation of medicinal plants is important in the discovery of novel drugs and also helps to asses toxicity risks associated with the use of either herbal preparations or conventional drugs of plant origin.

Today, as conventional medicine peruses a more integrated approach to managing disease, natural products and selected herbs that influence liver function are being revisited and evaluated for their overall health promoting effects. For developing satisfactory herbal combinations to treat severe liver disorders, plants have to be evaluated systematically for properties such as antiviral (hepatitis virus), antioxidant, stimulation of liver regeneration and choleretic activity. Single plants may not have all the desired activities. Even though herbal medicines are effective in the treatment of various ailments very often; these drugs are unscientifically exploited and or improperly used. Therefore these plant drugs deserve detailed studies in the light of modern science (Subramoniam and Pushpangadan, 1999).

*Eugenia jambolana* Syn. *Syzygium cumini* belong to Family Myrtaceae, commonly known as black berry, black plum (English), jamun or jambul (Hindi), naaval (Tamil), njaval (Malayalam) or Java plum, is a medium to large sized evergreen, glabrous tree with pale brown bark, slightly rough on old stems, which grows up to 30 m in height. It is distributed throughout India, Sri Lanka, Malaya and Australia. Leaves are opposite, simple, entire, elliptic-oblong, acute, smooth and shining. Several flavanoids, ellagitannins and phenolic acids have been identified from the fruits, seeds and aerial parts of *Eugenia jambolana* (Reynertson *et al.*, 2008). Various medicinal properties of *Eugenia jambolana* including its astringent, stomachic, diuretic and antidiabetic activities have been described in traditional medicine. Seeds are astringent, diuretic and strong antioxidants. Fruits and seeds are potent anti diabetic agents and can also be used to treat pharyngitis, spleenopathy, and ringworm infestations. It has several other medicinal properties in the folklore system of medicine. The bark of the plant is used as astringent, sweet, refrigerant, carminative, diuretic, digestive, anthelmintic, febrifuge, stomachic and antibacterial agent. Dried and powdered roots were used in diarrhoea and dysentery. The leaves were antibacterial and used to strengthen the teeth and gums and to treat constipation, burn wounds, vomiting, burning sensation in stomach and fever. Also it is used for poulticing in skin complaints and to inhibit blood discharges in the faeces. Phytochemical work on the leaf isolated the polyphenols including flavonoids, glycosides and phenolic acids and these were found strong antioxidants and free radical scavengers (Silva *et al.*, 2006). Lot of studies were conducted on the antidiabetic activity of the fruits and seeds and not much studies on the hepatoprotective property of the leaves of the plant.

The present study was therefore undertaken to evaluate the hepatoprotective effect of *Eugenia jambolana* leaves on paracetamol induced toxicity in rats. If found effective, it can be recommended for the protection of liver in various diseases of liver in field conditions.

# *Review of Literature*

# 2. REVIEW OF LITERATURE

## 2.1 PARACETAMOL

Dixon *et al.* (1971) examined the changes in the liver of rats sacrificed at various intervals after administration of large dose of paracetamol. It was found that the changes in liver were marked congestion, dilatation of central veins, disruption of surrounding sinusoids and frank necrosis of hepatocytes. He also opined that the histopathological changes of paracetamol over dosage were similar in man and rats.

Dixon *et al.* (1975) studied serum transaminase levels after experimental paracetamol induced hepatic necrosis and found that the dose of 4g/Kg paracetamol can produce hepatic necrosis and increase in alanine aminotransferase and aspartate aminotransferase after 24 hours of administration and reported that the serum enzyme levels gives a reliable indication of the severity of necrosis of liver.

Mogre *et al.* (1980) stated that paracetamol at the dose rate of 500mg/Kg orally twice a day for 7 days inhibited the sodium pump by interfering with sodium dependent phosphokinase in the reaction sequence of ATPase and there by caused liver injury.

Paracetamol at the dose rate of 2g/Kg orally produced reduction in bile flow, bile salts, total oxygen consumption and altered the biochemical parameters. The toxic metabolite of paracetamol (N- acetyl- *p* - benzoquinone imine -NAPQI) covalently bound to the receptor of hepatocyte membrane and disturbed the metabolic organizations there by reduced the viability of hepatocytes (Visen *et al.*, 1993).

Effect of acetaminophen on glutathione (GSH) S- transferase and related drug metabolizing enzymes was studied in vivo by Yonamine *et al.* (1996). They reported that acetaminophen caused oxidative stress and reduction in GSH content in liver homogenates at 1 hour after administration of acetaminophen and reached its minimum level at 3 hour.

Kaushal *et al.*(1999) evaluated the effects of paracetamol induced hepatotoxicity on microsomal functions and found that paracetamol at the dose of 650mg/ Kg intraperitoneally produced 6 fold increase in AST and 8 fold increase in ALT. It decreased the $Na^+$ -$K^+$ -ATPase activity and disturbed the $Ca^{2+}$ homeostasis and caused microsomal dysfunctions by impairing phospholipids metabolism. They also reported that paracetamol hepatotoxicity not affected the lipid peroxidation significantly.

Prescott (1999) observed that the liver damage caused by paracetamol is through a conversion by hepatic cytochrome P 450 enzymes to a toxic intermediate metabolite N-acetyl- *p*- benzoquinone imine (NAPQI) by a process called metabolic activation. Fasting increased the paracetamol hepatotoxicity in rats by decreasing glucuronide and sulphate conjugation so that the proportion converted to the toxic metabolite is significantly enhanced.

Udupa *et al.* (2000) reported that chronic administration of paracetamol at the dose rate of 500 and 1000 mg/ Kg for 4 weeks to rats produced dose dependent increase in ALT, AST and reduction in liver $Na^+$ $K^+$ ATPase activity, glycogen and glutathione levels indicating the hepatocellular damage. Histological examination showed evidence of swelling, hydropic degeneration and necrosis of the hepatocytes.

Asha (2001) stated that paracetamol over dosage (2g/Kg) damaged liver mainly by inducing lipid peroxidation directly or indirectly and also elevated the levels of Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT).

Paracetamol at the dose rate of 5 mmol/Kg intraperitoneally caused increase in plasma ALT, AST and glutamate dehydrogenase activity, without affecting bilirubin or creatinine concentration (Moore *et al.*, 2001).

Guzy *et al.* (2004) studied the effect of paracetamol on mitochondrial respiration, ATPase, glutathione peroxidase (GPX) and glutathione reductase (GR) activities and found that paracetamol in toxic doses (500mg/Kg) exhibited unfavorable effects on

respiration of mitochondria and on the activity of ATPase and not showed any significant effects on GPX and GR activity.

Kumar *et al.* (2004) stated that paracetamol at the dose rate of 3g/Kg orally produced the increase in levels of SGOT, SGPT, ALP and bilirubin and a decrease in total protein level.

Paracetamol at the dose rate of 3g/ Kg orally for three days produced elevation of serum marker enzymes like ALP, AST, ALT and lipid peroxides and caused a decrease in total protein and albumin. The hematological parameters were not much affected and on histological examination focal areas of necrosis were noted (Mathew, 2005).

Mohamed *et al.* (2005) reported that acetaminophen at the dose of 1g/Kg caused hepatotoxicity in rats and the toxic pathway is a cytochrome P- 450 dependent N-hydroxylation process in the liver and the hepatotoxicity of acetaminophen occurred when the liver enzymes catalyzing the normal conjugation reactions were saturated. Acetaminophen then metabolized and produced a toxic metabolite N –acetyl- *p*-benzoquinone imine (NAPQI-hydroxanic acid) which is then inactivated by conjugation with glutathione. A decrease in hepatic GSH to about 20% of initial concentration leads to covalent binding of acetaminophen metabolite to nucleophilic macromolecules in the cell and the toxic metabolites of acetaminophen induced cell damage in liver and other tissues such as kidney tubules.

Paracetamol at the dose rate of 200mg/ Kg orally produced the abnormal high level of serum ALT, AST, ALP and bilirubin which denoted the damage to the hepatic cells and also fatal hepatic necrosis in rats, man and mice. They also reported a reduction in total serum protein and liver glycogen which is associated with the decrease in the number of hepatocytes and decreased capacity to synthesize proteins and glycogen and consequently decreased the liver weight (Gupta and Misra, 2006).

Acetaminophen challenge (300mg/Kg, intraperitoneally) for 7 days caused significant increases in the levels of bilirubin, liver enzymes, thio barbituric acid reactive

substances (TBARS) and iron, while catalase activity and total protein levels were reduced significantly ( Iwalokun *et al.*, 2006).

Studies conducted by Ojo *et al.* (2006) revealed that paracetamol at the dose rate of 2g/Kg produced increase in serum levels of ALT, AST and ALP.

Studies conducted by Roy *et al.* (2006) revealed that paracetamol at the dose rate of 1g/Kg in three divided doses produced marked increase in serum levels of ALT,AST, ALP and bilirubin and it also produced severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis in liver.

Dash *et al.* (2007) reported that paracetamol at the dose rate of 750mg/ Kg resulted in a significant elevation of liver specific serum markers like ALT, AST, ALP, bilirubin and total protein and the levels of thiobarbituric acid reactive substances (TBARS) were considerably increased in rats. A decrease in the activities of glutathione, SOD and catalase were also noted.

## 2.2 HEPATOPROTECTIVE AGENTS

### 2.2.1 *Eugenia jambolana* / *Syzygium cumini*

Ethanolic extract of pulp of *Eugenia jambolana* at the dose rate of 100 and 200 mg/Kg orally for 10 days showed significant hepatoprotective activity in rats against paracetamol hepatotoxicity. It significantly decreased the elevated liver enzymes, increased the total protein and the effects are less when compared with standard drug silymarin (Gayatri and Das, 2006).

Hepatoprotective activity of *Eugenia jambolana* bark was reported by Sahu and Das (2006) in carbon tetrachloride ($CCl_4$) induced hepatotoxicity. They found that alcoholic extract of bark of *Eugenia jambolana* at the dose rate of 100 mg/Kg for 7 days offered significant protection to the liver by reducing the elevated serum enzymes levels.

Jasmine and Daisy (2007) studied the hypoglycemic and hepatoprotective activity of methanolic extract of *Eugenia jambolana* in Streptozotocin (STZ) - induced diabetic

rats. They found that oral administration of *Eugenia jambolana* extract at the dose of 150mg/Kg for 60 days to STZ induced diabetic rats decreased the blood glucose concentration and restored the normal functioning of liver. It also reduced the elevated levels of serum ALP, SGPT and SGOT in diabetic rats.

According to Moresco *et al.* (2007) the aqueous extract of *Syzygium cumini* at the dose rate of 0.9g/Kg orally for 7 days offered good protection against $CCl_4$ induced hepatotoxicity in rats. Single administration of 0.9g/Kg dose had no effect on toxicity. At the same, dose for 7 days reduced the increased serum AST and ALT activity. The scavenging of free radicals by the *Syzygium cumini* extract was responsible for the protective effect and it contained the flavonoids such as quarcetin, kaempferol and myricetin which inhibited the lipid peroxidation and there by offered the hepatoprotection.

### 2.2.2 Silymarin

According to Tedesco *et al.* (2004) the treatment with silymarin phytosome decreased the toxic severity of Aflatoxin $B_1$ (AF $B_1$). It reduced the increased serum ALT concentration in toxicity, retained the normal liver histology, feed intake and body weight gain in broilers. It can be used in chicken to prevent the effect of AF $B_1$ in contaminated feed.

Abrol *et al.* (2005) evaluated the different silymarin formulations both *in vivo* and *in vitro* and found that the hepatoprotective potential of silymarin in lipid microspheres was enhanced when tested in animal models like rat. It was also reported that phospholipids have a synergistic effect with silymarin.

Silymarin offered good protection in various toxic models of experimental liver diseases in laboratory animals and it acted by antioxidative, antilipid peroxidative, antifibrotic, antiinflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms (Pradhan and Girish, 2006).

Studies conducted by Dash *et al.* (2007) also revealed that silymarin is a flavolignan having hepatoprotective property. It is a mixture of three structural components silibinin, silydianine and silychristine. Silymarin is having hepatoprotective effects in acute viral hepatitis and intoxication with paracetamol, carbon tetrachloride and ethanol.

Silymarin, a flavolignan from *Silybum marianum* has excellent hepatoprotective action and its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on hepatocyte membrane, reduction of glutathione oxidation to enhance its level in the liver and intestine, antioxidant activity, stimulation of ribosomal RNA polymerase and subsequent protein synthesis leading to enhanced hepatocyte regeneration (Dixit *et al.*, 2007).

### 2.2.3 Other agents/ drugs having hepatoprotective activity

Natu *et al.*(1977) studied the effect of *Ricinus communis* leaves in experimental liver injury caused by carbon tetra chloride and found that whole leaves of *Ricinus communis* provided protection against liver necrosis as well as fatty changes induced by $CCl_4$, while the glycosidic and cold aqueous extract provided protection only against liver necrosis and fatty changes respectively. Because of the parasympathetic activity of these leaves, it increased the blood supply to the liver and there by offered protection against the hepatotoxins.

Mogre *et al.* (1980) studied the effects of *Picorrhiza kurroa* and *Eclipta alba* on $Na^+$ $K^+$ ATPase in hepatic injury by various hepatotoxic agents like carbon tetra chloride, paracetamol and aflatoxin. They reported that alcoholic extract of *P.kurroa* at the dose rate of 20mg/Kg once daily for seven days reduced the liver GOT and SGPT in all the three hepatic injuries and $Na^+$ $K^+$ ATPase levels were restored to normal. *E.alba* at the dose rate of 50mg/Kg for seven days also reduced the liver GPT in all hepatic injuries. But GOT content was reduced only in $CCl_4$ and paracetamol hepatic injury. $Na^+$ $K^+$ ATPase activity was raised in all the hepatic injury.

Singh *et al.* (1984) reported that *Tinospora cardifolia* provided protection to liver from the injurious effect of $CCl_4$ by the action of stabilizing the liver cell membrane and by protecting the enzyme related to rough endoplasmic reticulum. Administration of *T. cardifolia* before $CCl_4$ treatment significantly shortened the hexobarbitone and pentobarbitone induced sleeping time in $CCl_4$ treated rats.

Agrawal *et al.*(1986) conducted screening of *Phyllanthus niruri* and *Ricinus communis* on alcohol induced liver cell damage in non- hepatectomised and partially hepatectomised rats and found that 50% alcoholic extract of roots and leaves of *P.niruri* has got hepatoprotective effects on alcohol induced liver cell damage. The alcoholic extract of *Ricinus communis* did not show any significant hepatoprotective activity.

Gulati *et al.* (1991) reported that ethanolic extract of roots of *Boerhaavia diffusa* at the dose rate of 100 mg/100g body weight/day for 21 days offered significant hepatoprotection in country made liquor hepatotoxicity. It normalized the level of ALT, triglycerides and cholesterol in extract treated animals.

Chattopadhyay *et al.* (1992) studied the effect of *Ocimum sanctum* leaf extract on paracetamol induced hepatic damage in rats and found that *O.sanctum* protected the rats from the hepatotoxic action of paracetamol as evidenced by significant reduction in the elevated serum levels. Histological studies also showed marked reduction in fatty degeneration in animals received the extract.

According to Chattopadhyay *et al.*(1992), water soluble portion of alcoholic extract of *Azadirachta indica* at the dose rate of 1g/Kg orally for seven days produced marked decrease in levels of ALT, AST, ALP and acid phosphatase in paracetamol induced hepatotoxicity. It also prevented the necrotic changes in the liver.

Murti and Srinivasan (1993) stated that *Tephrosia purpurea* at 500 mg/Kg dose level offered hepatoprotection in D- Galactosamine and $CCl_4$ induced toxicity. It decreased the serum marker enzymes like SGPT, SGOT and bilirubin in hepatotoxicity induced by D- Galactosamine and $CCl_4$.

A synthetic agent 89/62 (3 cyano-4 –Methyl -5- Vinyl Pyridine) which is structurally similar to Ricinine from the *Ricinus communis* produced a marked increase in bile flow, bile salts, bile acids like cholic acid and deoxycholic acid in the bile.It also normalized the serum marker enzymes in various toxic agents and thereby acting as hepatoprotective (Visen *et al.*,1993).

Ursolic acid which is isolated from leaves of *Eucalyptus tereticomis* have significant hepatoprotective activity in thioacetamide, galactosamine and $CCl_4$ induced hepatotoxicity in rats (Saraswat *et al.*, 1996). Pretreatment with ursolic acid increased the viability of rat hepatocytes. It also showed anticholestatic acitivity in a dose dependent manner.

N Acetyl Cysteine (NAC) at the dose rate of 400mg/Kg has hepatoprotective property by its ability to enhance the glutathione synthesis there by providing more substrate for the detoxification of the hepatotoxic metabolite of paracetamol (Al-Mustafa *et al.*, 1997).

Jayasekhar *et al.* (1997) stated that at the dose rate of 250mg/ Kg the ethyl acetate extract of *Acacia catechu* offered significant hepatoprotection by lowering the increased level of serum transaminase, serum ALP and serum bilirubin in $CCl_4$ treated rats.

Rao and Misra (1997) studied the effects of different parts of *Sida rhombifolia* on chemical and drug induced hepatotoxins like paracetamol, Carbon tetra chloride, rifampicin and on carrageenan induced paw edema in rats. It was found that the powdered roots, aerial parts and their aqueous extract showed significant hepatoprotective activity and methanolic extract of aerial parts have significant edema suppressant activity in rats.

Saraswathy *et al.* (1998) evaluated the effect of Liv.100 against the anti - tubercular drugs (Isoniazid, rifampicin and pyrozinamide) induced hepatotoxicity in rats. At the dose rate of 400mg/Kg, Liv.100 offered sufficient hepatoprotection to rats. Simultaneous administration of Liv.100 showed near normal levels of marker enzymes, and the levels of lipid peroxides, glutathione content on comparison with normal control.

Ahmad *et al.* (1999) studied the hepatoprotective potential of Jigrine, which is a polyherbal preparation containing 14 medicinal plants on thioacetamide induced hepatotoxicity and reported that pretreatment with Jigrine reduced the elevated levels of AST, ALT, $Na^+$ and $K^+$ levels in serum and TBARS. Its effects on tissues were similar to Silymarin which was recognized as a standard hepatoprotective drug.

Al- Sereiti *et al.* (1999) reported that the lyophilized ethanol and aqueous extracts of young sprouts of *Rosemarinus officinalis* (Rose Mary) have choleretic activity and offered protection against $CCl_4$ induced toxicity in rats. It caused a significant increase in bile flow and a significant reduction in elevated plasma liver enzymes.

Aqueous and alcoholic extract of *Cassia occidentalis* at the dose rate of 500mg/ Kg orally for 7 days provided sufficient hepatoprotection in paracetamol and ethyl alcohol intoxication in rats (Jafri *et al.*, 1999).

Trivedi and Rawal (2000) conducted hepatoprotective and toxicological evaluation of *Andrographis paniculata* on severe liver damage caused by BHC (hexa-chloro cyclohexane). They found that *Andrographis paniculata* at the dose of 12mg/Kg provided protection by decreasing the elevated levels of ALP, AST, ALT and gamma GPT and decreased the level of lipid peroxidase.

Udupa *et al.* (2000) evaluated the effect of HD-03, a polyherbal preparation which consists of *Phyllanthus niruri, Cichorium intybus, Emblica officinalis, Tephrosia purpurea* and *Andrographis paniculata* on paracetamol induced liver damage. They reported that at the dose rate of 750mg/Kg for 28 days provided sufficient hepatoprotection by reversal of $Na^+$ $K^+$ ATPase, glycogen, glutathione levels and restricted the liver damage by the paracetamol.

Asha (2001) studied the hepatoprotective activity of *Marmodica subangulata* and found that administration of tender leaves at the dose rate of 50mg/Kg markedly prevented paracetamol induced elevation of serum enzymes such as ALT, AST and ALP.

*Ginkgo biloba* at the dose of 50mg/Kg intraperitoneally for 7 days decreased the elevated levels of AST, ALT, ALP and increased the total protein and albumin levels in treated animals against $CCl_4$ induced toxicity and reported that *Ginkgo biloba* protected the liver from $CCl_4$ induced liver damage(Shenoy *et al.*,2001).

The hepatoprotective effects of *curcuma longa* rhizomes against paracetamol toxicity were evaluated by Somchit *et al.* (2002). He reported that pretreatment of rats with ethanolic extract of *curcuma longa* at the dose of 100mg/Kg prior to paracetamol dosing statistically lowered the three serum marker enzymes like AST, ALT and ALP and thereby have hepatoprotective effect.

Ethanolic and butanolic extract of fruits of *Piper longum* at the dose rate of 300mg/Kg orally for 4 days offered hepatoprotection in $CCl_4$ induced hepatotoxicity by decreasing the elevated SGPT and SGOT activity (Jalalpure *et al.*, 2003)

According to the studies conducted by Gupta *et al.* (2004) the methanol extract of the plant *Bauhinia racemosa* at the dose rate of 50, 100 and 200 mg/ Kg produced significant hepatoprotective effect on both paracetamol and $CCl_4$ toxicity by decreasing the activity of serum marker enzymes like ALP, AST, ALT, and bilirubin and lipid peroxidation. It significantly increased the levels of GSH, SOD, CAT and protein in a dose dependent manner and it also has antioxidant effects on ferric chloride- ascorbate-induced lipid peroxidation in rat liver homogenate.

Guzy *et al.*(2004) reported that quarcetin (3, 3' 4' ,5, 7 Penta hydroxy flavone) is the major bioflavonoid in tea, onion, and red wine and which offered a potential for ameliorating the hepatotoxicity of paracetamol by its antioxidant effect and it prevented the paracetamol induced decrease in oxygen consumption.

Hewawasam *et al.*(2004) found that pre-treatment of *Epaltes divaricata* extract at the dose rate of 0.9g/Kg orally for 7 days significantly reduced the elevated serum levels of ALT, AST, ALP and significantly increased the liver reduced glutathione in $CCl_4$ induced hepatotoxicity.

Kumar *et al.* (2004) evaluated the effect of ethanolic extract of *Trianthema portulacastrum* was studied against paracetamol induced hepatotoxicity in rats. Treatment of rats with a dose rate of 200mg/Kg orally for 10 days significantly reduced the increased levels of ALT, AST, ALP and bilirubin. It significantly increased the total protein

Maheswari and Rao (2005) studied the effect of oral administration of grape seed oil (GSO) against $CCl_4$ induced hepatotoxicity in rats and found that oral administration of GSO (3.7g/Kg) for seven days resulted in a significant reduction in serum AST, ALT, ALP levels, liver MDA and hydroperoxidases. A significant improvement of glutathione, SOD, catalase and total protein were also noted. The histological changes caused by $CCl_4$ are also brought back to normalcy in drug co- administered rats.

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 400mg/Kg significantly reduced the elevated liver enzymes, there by showed hepatoprotective action. They also stated that the flavanoids present in the plant as well as its antioxidant property were responsible for the hepatoprotective action.

Mankani *et al.* (2005) reported the hepatoprotective activity of ethanolic extract of stem bark of *Pterocarpus marsupium* and they described that at the dose rate of 25mg/ Kg/day orally for 14 days, the toxic effect of $CCl_4$ was controlled significantly as evidenced by restoration of the levels of serum bilirubin, proteins and enzymes to the normal level.

Mathew (2005) conducted studies on hepatoprotective effect of *Aegle marmelos* and *Azadirachta indica* aqueous leaf extract on paracetamol toxicity in rats and found that *Azadirachta indica* at the dose of 500mg/Kg orally for 12 days and *Aegle marmelos* at the rate of 1g/Kg orally for 12 days offered good protection by reducing the liver serum marker enzymes.

The methanolic extract of the plant *Berberis tinctoria* at the doses of 150 and 300 mg/Kg produce significant hepatoprotective effect on paracetamol induced hepatic

damage in rats by decreasing the activity of serum enzymes like ALT, ALP, AST, bilirubin and lipid peroxidation. It significantly increased the levels of glutathione (GSH), Catalase (CAT) and superoxide dismutase (SOD) in a dose dependent manner and these were having good antioxidant property. These effects were comparable with the standard hepatoprotective drug silymarin (Murugesh *et al.*, 2005).

Oyejide and Olushola (2005) studied the hepatoprotective and antioxidant effects of extract of *Carmellia sinensis* (black tea) in rats against the sodium oxalate toxicity. They reported that at the dose rate of 100mg/ Kg, it significantly lowered the serum and tissue levels of Malondialdehyde as well as AST and ALT activities in a dose dependent manner. The result also indicated that prolonged tea administration at the dose rate of 200mg/Kg body weight for 20 days significantly increased serum Vitamin C level and the activity of catalase in the serum, liver and kidney.

Studies conducted by Tabassum *et al.* (2005) revealed that *Phyllanthus niruri* extract at the dose rate of 100mg/100g /day for five consecutive days offered sufficient hepatoprotection in paracetamol induced hepatotoxicity in mice and it was assessed by estimating the liver marker enzymes.

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* orally in two doses 20 and 40 mg/Kg/day significantly reduced the elevated levels of ALT, AST and ALP in toxicity and the administration of extract effectively inhibited the fatty changes in the liver. They also reported that the administration of asiaticoside significantly increased the level of antioxidant enzymes like SOD, catalase and glutathione peroxidase.

Gupta and Misra (2006) reported the hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol intoxication in rats. Administration of the plant extract increased the concentration of glutathione in blood and liver and liver $Na^+$ $K^+$ ATPase activity. Treatment with aqueous ethanolic extract of *C .recutita* reduced the enhanced level of serum ALT, AST, ALP and bilirubin. It acted

as a free radical scavenger and increased the GSH in blood and by its antioxidant effect. They also reported that the hepatoprotective activity of the powdered drug and its extract was due to the stimulatory effects on hepatic regeneration or free radical scavenging effects. The edema suppressant activity of the drug was due to the inhibitory effects on release of histamine like substances.

Iwalokun *et al.*(2006) evaluated the hepatoprotective and antioxidant effects of an aqueous extract of *Vernonia amygdalina* leaves against acetaminophen induced hepatotoxicity and oxidative stress in mice *in vivo*. They reported that pre administration of *V. amygdalina* resulted in a dose dependent (50-100mg/kg) reversal of acetaminophen-induced alterations of all the liver function parameters and suppression of acetaminophen induced lipid peroxidation and oxidative stress.

According to Jain *et al.* (2006) ethanol extract of leaves of *Tephrosia purpurea* and flavanoid isolated from leaf extract offered sufficient hepatoprotection in $CCl_4$ induced hepatotoxicity in rats at the dose rate of 100mg/Kg/day for 4 days. It caused a decrease in SGOT, SGPT, SALP and total bilirubin to normal level.

Kumar *et al.* (2006) evaluated the protective effect of root extract of *Operculina turpethum* against paracetamol induced hepatotoxicity in rats and found that the ethanolic extract obtained from the plant at a dose rate of 200mg/Kg has significant hepatoprotective effect by lowering serum levels of SGOT, SGPT, ALP and total bilirubin. It can prevent the paracetamol induced hepatic necrosis also.

Olaleye *et al.* (2006) discovered that an African plant *Alchornea cordifolia* have significant protection against hepatotoxic, necrotic and peroxidative actions of acetaminophen and it prevented the accumulation of toxins in liver and inhibited the formation of peroxides at a dosage between 300-500 mg/ Kg of the extract. It normalized the elevated serum enzymes in paracetamol toxicity.

Ozbek *et al.* (2006) reported that the *Foeniculum vulgare* fixed oil (FFO) offered sufficient hepatoprotection in $CCl_4$ induced liver fibrosis in rats. The FFO at the dose rate

of 0.2 mg/Kg three times a week for 7 days reduced the increased levels of ALT, AST, ALP and bilirubin.

*Psidium guajava* leaf extract at the dose level of 500mg/ Kg orally significantly reduced the elevated serum levels of AST, ALT, ALP and bilirubin. It prevented the increase in liver weight in paracetamol toxicity. It gave protection against $CCl_4$ and thioacetamide induced hepatotoxicity. This plant also showed antibacterial, antidiorrhoeal, hyperglycaemic and antioxidant activities in addition to the hepatoprotective effect (Roy *et al.,* 2006).

Antioxidant and protective effect of an Oleanolic acid enriched extract of *Actinidia deliciosa* (Chinese gooseberry) root on $CCl_4$ induced liver injury in rat was studied by Bai *et al.* (2007). They reported that at 120mg/ Kg dose, ethanol water extract of this plant showed high anti oxidant activity in vitro and hepatoprotective property in vivo when analyzed by methods of Ferric thiocyanate and thiobarbituric acid. It reduced the elevated serum marker enzymes and lipid peroxidation, GSH concentration were increased.

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 at the dose rate of 200 and 300 mg/Kg produced significant hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin, uric acid, lipid peroxides. It also significantly increased the levels of SOD, CAT, GSH and protein in a dose dependent manner.

Chloroformic and methanolic extract of the plant *Ichnocarpus frutescens* at a dose level of 250mg/ Kg and 500mg/ Kg offered significant hepatoprotection by decreasing the activity of serum enzymes, bilirubin and lipid peroxidase. These significantly increased the levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in a dose dependent manner in paracetamol toxicity and these results were comparable with standard hepatoprotective drug silymarin. These have potent anti oxidant effects also (Dash *et al.*, 2007).

Gujrati *et al.* (2007) investigated the hepatoprotective activity of alcoholic and aqueous extract of leaves of *Tylophora indica* against ethanol induced hepatotoxicity. They found that pretreatment with alcoholic extract or aqueous extract significantly prevented the physical, biochemical, histological and functional changes induced by ethanol in liver at the dose level of 200 and 500 mg/Kg alcoholic extract and 125 and 300 mg/ Kg aqueous extract.

Kumar and Misra (2007) studied the hepatoprotective effect of acetone and ethanolic sub fractions of the plant extract of *Pergularia daemia* at the dose of 100 and 150 mg/Kg orally and found that the extract was able to reduce all the elevated biochemical parameters like SGPT, SGOT, and ALP. They also reported that the flavanoids which were present in the extract was responsible for the hepatoprotective effect.

Mir *et al.* (2007) reported that the alcoholic extract of *Emblica officinalis* at the dose rate of 100mg/Kg orally for 8 weeks reversed the $CCl_4$, paracetamol and thioacetamide induced abnormal histopathology on liver cells by its ability to accelerate the regenerative activity.

The methanol extract of the leaves of *Ficus carica* was evaluated for its hepatoprotective activity in rats with liver damage induced by $CCl_4$. It was reported that the extract at an oral dose of 500mg/ Kg exhibited a significant protective effect by lowering the increased serum levels of ALT, AST, total bilirubin and malonaldehyde equivalent and an index of lipid peroxidation in liver (Mohan *et al.*, 2007).

Pimple *et al.* (2007) reported the hepatoprotective effect of fruits and leaves of *Tamarindus indica* at the dose rate of 350mg/ Kg .It reduced the serum enzyme levels of ALT, AST and ALP in paracetamol induced hepatotoxicity.

Sengottuvelu *et al.* (2007) conducted studies on hepatoprotective activity of *Cleome viscosa* in $CCl_4$ induced hepatotoxicity. They reported that aqueous seed extract of *Cleome viscosa* at the dose rate of 200mg/Kg administered orally for 7 days , made reduction in serum ALT, AST , ALP ,Gamma – Glutamyl transpeptidase and lipid

peroxidase and increased the reduced glutathione(GSH) in the treated animals and histological changes were also prevented by extract.

Sundaram and Mitra (2007) reported about the antioxidant activity of ethyl acetate soluble fraction of *Acacia arabica* bark in rats and they reported that the polyphenol rich active fractions of *Acacia arabica* is a potent free radical scavenger and there by it showed hepatoprotective effect at the dose rate of 150 mg/Kg for 19 days in carbon tetrachloride induced toxicity.

## 2.3 OTHER PHARMACOLOGICAL EFFECTS OF *EUGENIA JAMBOLANA / SYZYGIUM CUMINI*

According to Gupta and Sharma (1974) the *Eugenia jambolana* leaves contained triterpenoids, n paraffins, aliphatic alcohols, sitosterol, butulinic acid, categolic acid and sugars like glucose and fructose.

Bansal *et al.* (1981) observed that oral administration of seeds of *Eugenia jambolana* in rats for 15 days caused a marked lowering of blood glucose levels (BGL) accompanied with a significant increase in pancreatic cathepsin B activity and the effects were compared to those of chlorpropamide when fed under same condition.

Rajasekharan *et al.* (1988) evaluated the antifertility activity of oleanolic acid isolated from the flowers of *Eugenia jambolana* in male albino rats. It was reported that the administration of the compound at the dose of 30 mg/Kg for 60 days decreased the fertilizing capacity of animals without any significant changes in body weight or reproductive organ weights and caused arrest of spermatogenesis.

The aqueous extract of *Eugenia jambosa* fruit pulp is highly acidic and is responsible for the astringent action according to the studies conducted by Noomrio and Dahot (1996). They evaluated the nutritive value of this fruit and found that it is rich in minerals such as sodium, potassium and calcium. It also contained phosphorus, iron, manganese, magnesium and many vitamins. A significant amount of soluble protein, total sugar and reducing sugars were also present in the aqueous extract.

Grover *et al.* (2000) studied the antihyperglycemic effect of *Eugenia jambolana* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. At the dose of 200mg/Kg/day of lyophilized powder restored the alteration in hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexakinase, glucose -6- phosphate and phospho fructokinase levels in diabetic mice in mild to moderate hyperglycemia. It has no effect on haematological parameters and their mechanism of action was similar to sulfonylurea and biguanides.

The subchronic oral administration of fresh *Eugenia jambolana* leaf decoction to STZ -induced diabetic rats was not useful for the treatment of diabetes (Pepato *et al.*, 2001). There were no alterations in serum triglyceride levels or adipose tissue weight in STZ - diabetic rats which indicated that the treatment didnot improve lipid metabolism.It has no protective effect on blood vessels and did not improve the protein metabolism in diabetic rats.

Jagetia and Balinga (2002) reported about the effects of various concentrations of leaf extract of *Syzygium cumini* in radiation induced DNA damage in the cultured human peripheral blood lymphocytes. They stated that the leaf extract of *Syzygium cumini*, at the dose rate of 25-100µg/ml protected the cells against the radiation induced DNA damage.

Muruganandan *et al.* (2002) evaluated the anti- inflammatory activity of *Syzygium cumini* bark against inflammation induced by autacoids like histamine, serotonin, bradykinin and Prostaglandin $E_2$ and reported that it has inhibitory role on inflammatory response to all, except the bradykinin.

Timbola *et al.* (2002) reported about a new flavonol myricetin from the leaves of *Eugenia jambolana* and the leaves were contained triterpenoids, essential oils and quarcetin.

Ethanolic extract of seeds of *Eugenia jambolana* exhibited significant hypoglycemic effect in mild and severe diabetic rabbits after 15 days treatment at the dose of 100 and 200 mg/Kg. The active hypoglycemic compound present in the ethanolic extract of seeds did not require the presence of functioning β cells for the favorable action

seen in type I and II diabetes. These caused reduction in glycosylated haemoglobin in mild and severe diabetes in rabbits. *Eugenia jambolana* seeds also prevented the complications of lipid profile seen in diabetes in which hyperglycemia and hypercholesteremia co exists (Sharma *et al.*, 2003)

Ravi *et al.* (2004) investigated the effect of *Eugenia jambolana* seed kernel on antioxidant defense system of plasma and pancreas in STZ - induced diabetic rats. A significant increase in the levels of plasma glucose, vitamin E, ceruloplasmin, lipid peroxides was noted by them. A concomitant decrease in the levels of Vitamin C, reduced glutathione which were observed in diabetic rats were reverted back to near normal level after the treatment with seed kernel extract at the rate of 100mg/ Kg/ day for 30 days. Presence of flavanoids was responsible for the protective antioxidant effect.

According to Banarjee *et al.* (2005) the antioxidant property of fruit skin of *Eugenia jambolana* was due to the presence of anthocyanins, antioxidant vitamins, phenolics or tannins present in them and they can prevent the lipid peroxidation.

Indrayan *et al.* (2005) reported about the nutritive value and mineral contents of the plant *Eugenia jambolana* and opined that it is rich in magnesium and iron, poor in calcium and has moderate protein value. They also reported that the seeds were having astringent, diuretic action and can stop urinary discharges.

Streptozotocin (STZ) -induced diabetic rats when administered with 50mg/ day of lyophilized *Eugenia jambolana* fruit pulp extract for 41 days showed no observable difference in body weight, food or water intake, urine volume, glycaemia, urinary urea, glucose, hepatic glycogen or serum levels of total cholesterol, HDL cholesterol or triglycerides. No detectable changes were observed in masses of epidydymal or retroperitoneal adipose tissue (Pepato *et al.*, 2005).

Ravi *et al.* (2005) reported the antihyperlipidemic effect of *Eugenia jambolana* seed kernel on STZ -induced diabetic rats. The effect of oral administration of ethanolic extract of *Eugenia jambolana* seed kernel (100mg/ Kg body weight) was examined on the levels of cholesterol, phospholipids, triglycerides and free fatty acids in the plasma,

liver and kidney tissues of diabetic rats. The plasma lipoproteins (HDL, LDL, VLDL-cholesterol) and fatty acid composition were altered in STZ diabetic rats and these levels were reverted back to near normalcy by *Eugenia jambolana* extract. It has hypolipidemic effect, which was due to the flavonoids, saponins, glycosides and triterpenoids in the extract.

The antidiabetic effect of *Eugenia jambolana* seed powder was found good at the dose rate of 500mg/ Kg for 15 days. It caused an increase mean body weight, reduced the BGL and elevated the liver glycogen values (Sridhar *et al.*, 2005).

Mallick *et al.* (2006) studied the antihyperglycemic and antihyperlipidemic effects of separate and composite extract of seed of *Eugenia jambolana* and root of *Musa paradisiaca* in STZ- induced diabetic male albino rats. They found that methanolic extract of the plants in separate as well as in composite manner in diabetic rats resulted in a significant remedial effect on blood glucose level (BGL) as well as in the quantity of liver and skeletal muscle glycogen. The serum lipid profiles were also corrected significantly in diabetic rats after the treatment. The composite extract is found more effective than the separate extract according to them.

Effect of jaman fruit (*Eugenia jambolana)* fruit extract on serum lipid profile in Type II diabetic individuals were studied by Safdar *et al.* (2006). Ninety ml of extract in three divided doses produced a decrease in serum glucose, total cholesterol, LDL and the HDL were not much affected.

*Eugenia jambolana* was reported useful as digestive astringent to the bowels, anthelmintic, anti diabetic, antibacterial, analgesic, anti-inflammatory, antioxidant and gastro protective (Sagrawat *et al.*,2006).

*In-vitro* antiviral activity of *Eugenia jambolana* aqueous leaf extract on buffalo pox virus was evaluated by Bhanuprakash *et al.* (2007) and reported that these were very effective against buffalo pox virus. The leaves of *Eugenia jambolana* contained several polyphenolic compounds and flavanoids which were responsible for these activities.

Brito *et al.* (2007) investigated the antiallergic properties of an aqueous leaf extract of *Syzygium cumini* and found that oral administration of *Syzygium cumini* at the dose of 25-100mg/Kg in Swiss mice inhibited the paw edema and prevented the mast cell degranulation and consequent histamine release.

Chaturvedi *et al.* (2007) reported that ethanolic extract of *Eugenia jambolana* at the dose rate of 200mg/Kg, when administered orally for 10 days in rats was found to reduce the ulcer index in all gastric ulcer models. It tended to decrease acid pepsin secretion, cell shedding enhanced mucin and mucosal glycoprotein and had no effect on cell proliferation.

Jasmine and Daisy (2007) studied the hypoglycemic and hypolipidemic activity of *Eugenia jambolana* in STZ- diabetic rats at the dose of 150mg/Kg/day for 60 days. Serum glucose concentration, total cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides were decreased and at the same time serum insulin and HDL cholesterol levels markedly increased.

Jasmine *et al.* (2007) had done studies on *in vitro* efficacy of flavonoids from *Eugenia jambolana* seeds against Extended Spectrum β Lactamase (ESβL) producing multidrug resistant enteric bacteria. They found that most Extended Spectrum β Lactamase positive strains were resistant to several antibiotics. However, these were sensitive to this plant extract and the flavonoids present in them were responsible for the antibacterial activity.

Acute toxicity studies of *Syzygium cumini* seed extract proved that up to 2000mg/Kg dose rate has no toxic effect on animals. The ethyl acetate and methanolic extract of *Syzygium cumini* has CNS depressant activity in mice by potentiating the concentration of GABA in brain due to the presence of saponins (Kumar *et al.*, 2007).

MTEC, a formulated herbal drug consists of *Musa paradisiaca, Tamarindus indica, Eugenia jambolana* and *Coccinia indica* at the dose level of 60mg/day, two times a day for 14 days offered protection of testicular dysfunctions in STZ- induced diabetic rats. It gave a significant protection in fasting blood glucose level (BGL) and serum

insulin levels along with correction of testicular dysfunctions like decreased sperm count, viability and motility and androgen synthesis. It has no general toxic effects on body weight as well as on the SGPT and SGOT in the serum (Mallick *et al.*, 2007).

The anti microbial activity of *Syzygium cumini* leaves extract was evaluated by Oliveira *et al.* (2007) and found that crude hydro alcoholic extract was active against Candida, multi resistant strains of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus.* The anti microbial activity was due to the presence of tannins and other phenolic constituents and it was rich in ellagic acid polyphenol derivatives and gallic acid.

Sultana *et al.* (2007) evaluated the antioxidant activity of phenolic components present in the bark of *Azadirachta indica, Eugenia jambolana, Terminalia arjuna* and *Acacia nilotica* and proved that *Eugenia jambolana* has the maximum antioxidant activity out of four of these plants.

Chemical nature of anthocyanins present in the berries of *Syzygium cumini* was reported by Veigas *et al.* (2007) and they reported that the high antioxidant activity of the extract at extremely low concentrations makes *Syzygium cumini* a potential source of anti oxidants as well as a natural colourant.

Sharma *et al.* (2008) studied the effects of flavanoids rich extract from seeds of *Eugenia jambolana* (L.) on carbohydrate and lipid metabolism in diabetic mice. They reported that various biochemical parameters like glucose tolerance, lipid profile, glycogen biosynthesis, glucose uptake and insulin release were improved significantly both *in-vivo* and *in-vitro.* They also opined that the extract has hypoglycemic, hypolipidemic and antioxidant properties.

# *Materials and Methods*

# 3. MATERIALS AND METHODS

## 3.1 EXPERIMENTAL ANIMALS

The study was conducted in 40 adult male Wistar rats weighing150- 200 g. The rats were purchased from Small Animal Breeding Station, Mannuthy. The animals were housed in appropriate cages in a well ventilated room with a 12-hour light: 12-hour dark cycle. They were maintained under identical feeding and management practices in the laboratory. An acclimatization period of seven days was allowed before the commencement of the experiment. The experiment was conducted for a period of 10 days.

## 3.2 PLANT MATERIALS

The leaves of the plant of *Eugenia jambolana* was collected from the campus of College of Veterinary & Animal Sciences, Mannuthy, Thrissur district, Kerala and identified (Fig.1).

### 3.2.1 Preparation of alcoholic extract of *Eugenia jambolana*

The leaves of the plant *Eugenia jambolana* was air-dried under shade and coarsely powdered using an electrical pulverizer. The powder obtained was extracted using a Soxhlet apparatus with 95% ethanol. The ethanolic extracts were then concentrated in a rotary vacuum evaporator under reduced pressure and temperature (55°C) and kept under refrigeration for the complete evaporation of the solvent. The yield of the extracts was 23.4 % on dry matter basis.

## 3.3 PHYTOCHEMICAL SCREENING

The ethanolic extract of leaves of plant of *Eugenia jambolana* was tested for the presence of various active chemical constituents namely steroids, alkaloids,

Fig. 1.*Eugenia jambolana* leaves

phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins (Harborne, 1991). Tests for nitrate (Householder *et al.*, 1966) and cyanide (Bark, 1963) were also conducted.

### 3.3.1 Tests for Detection of Steroids

#### *3.3.1.1 Salkowski test*

About 5mg of the extract was mixed with 3ml of chloroform and then shaken with 3ml concentrated sulphuric acid. Development of red colour indicates the presence of steroids.

#### *3.3.1.2 Liberman Burchardt test*

About 5mg of the extract was mixed with 3ml of chloroform in a test tube. Then five drops of acetic anhydride and 1ml of concentrated sulphuric acid were added to it through the sides of the test tube. Development of a reddish ring at the junction of two layers indicates the presence of steroids.

### 3.3.2 Tests for Detection of Alkaloids

About 0.5g of the extract was mixed with 5ml of ammonia and then extracted with equal volume of chloroform. To this, 5ml dilute hydrochloric acid was added. The acid layer obtained was used for the following chemical tests for alkaloids.

#### *3.3.2.1 Mayer's test (potassium mercuric iodide)*

To 1 ml of acid layer, a few drops of Mayer's reagent (1.358g of mercuric chloride dissolved in 60 ml of water and poured into a solution of 5g of potassium iodide in 10ml of water and then make up the volume to 100ml with distilled water)

were added. Development of a creamy white precipitate indicates the presence of alkaloids.

*3.3.2.2 Wagner's test*

A few drops of Wagner's reagent (2g of iodine and 6g of potassium iodide dissolved in 100ml of water) were added to 1ml of the acid extract. Development of reddish brown precipitate indicates the presence of alkaloids.

*3.3.2.3 Hager's test (saturated solution of picric acid)*

To 1ml of the acid extract, a few drops of Hager's reagent (1g of picric acid dissolved in 100ml of water) were mixed. Development of yellow precipitate indicates the presence of alkaloids.

*3.3.2.4 Dragendroff's test*

A few drops of Dragendroff's reagent (Stock solution (1) 0.6 grams of bismuth sub nitrate was dissolved in 2ml of concentrated hydrochloric acid and 10ml of water was added. Stock solution (2) six grams of potassium iodide was dissolved in 10ml of water. Then both the stock solutions (1) and (2) were mixed together and then it was mixed with 7ml of concentrated hydrochloric acid and 15ml of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 400ml) and were mixed with 1ml of acid extract. Development of a reddish brown precipitate indicates the presence of alkaloids.

**3.3.3 Test for Detection of Phenolic compounds**

About 5mg of the extract was dissolved in 1ml of water and five drops of ten per cent ferric chloride was added to it. Development of dark blue colour indicates the presence of phenolic compounds.

### 3.3.4 Tests for Detection of Tannins

#### *3.3.4.1 Ferric chloride test*

Two milligram of the extract was mixed with 3ml of one per cent ferric chloride solution. Development of a blue, green or brownish colour indicates the presence of tannins.

#### *3.3.4.2 Gelatin test*

About 0.5g of the extract was mixed with a few drops of one per cent solution of gelatin containing ten per cent sodium chloride. Development of a white precipitate indicates the presence of tannins.

### 3.3.5. Tests for Detection of Flavonoids

#### *3.3.5.1 Ferric chloride test*

To 2ml of alcoholic solution of the extract (0.5g extract in 10ml methanol), a few drops of neutral ferric chloride solution was mixed. Development of green colour indicates the presence of flavonoids.

#### *3.3.5.2 Lead acetate test*

To 2ml of alcoholic solution of the extract (0.5g extract in 10ml methanol), a few drops of neutral ten per cent lead acetate was mixed. Development of a yellow precipitate indicates the presence of flavonoids.

### 3.3.6 Tests for Detection of Glycosides

#### *3.3.6.1 Sodium hydroxide test*

A small amount of the extract (about 5mg) was mixed with 1ml water and 5-6 drops of sodium hydroxide solution (10%) were added. Development of a yellow colour indicates the presence of glycosides.

#### *3.3.6.2 Benedict's test*

To about 1ml of the extract (0.5g extract in 1ml of water), 5 ml of Benedict's reagent was added. The mixture was boiled for two minutes and cooled. Development of brown to red colour indicates the presence of glycosides.

### 3.3.7. Test for Detection of Diterpenes

About 5mg of the extract was mixed with 3ml of copper acetate solution (5 per cent). Development of green colour indicates the presence of diterpenes.

### 3.3.8. Tests for Detection of Triterpenes

#### *3.3.8.1 Salkowski test*

About 3mg of the extract was mixed with 3ml of chloroform and then it was shaken with 3ml of concentrated sulphuric acid. Development of yellow colour in lower layer on standing indicates the presence of triterpenes.

#### *3.3.8.2 Lieberman Burchardt test*

A few drops of acetic acid and 1ml concentrated sulphuric acid were added to 3 ml of chloroform solution of the extract (about 3mg extract in 3ml chloroform).

Development of deep red ring at the junction of two layers indicates the presence of Triterpenes.

### 3.3.9 Test for the Detection of Saponins

*Foam test*

A small amount of the extract (about 5mg) was shaken with 3ml of water. Development of the foam that persists for ten minutes indicates the presence of saponins.

### 3.3.10 Test for the detection of nitrates

The plant material was tested with diphenylamine reagent (Diphenylamine stock solution was prepared by dissolving 0.5 g of diphenylamine in 20 ml of water and concentrated sulphuric acid was added to bring the volume to 100 ml. equal part of this stock solution was mixed with 80% sulphuric acid.) The test was conducted by adding few drops of reagent to the crushed plant material. A bright blue colour indicates presence of nitrate (Householder *et al.*, 1966).

### 3.3.11 Test for the detection of cyanides

Finely chopped fresh plant materials (5g) were added to 100ml boiling tube. 4-12 drops of chloroform was added. To the stoppered lid, a picrate paper was anchored in such a way that this picrate paper was hanging away from the bottom of the liquid. The test tube was kept on a water bath at 30-37$^0$ C for about 3 hours. If the test is positive, yellow picrate paper will turn to red (Bark, 1963).

## 3.4 EXPERIMENTAL DESIGN

The animals were randomly divided into 5 groups comprising eight animals each. The experiment was conducted for a period of 10 days. Blood was collected from all the groups prior to the experiment for haematological and biochemical studies.

Group I – Healthy control was administered with vehicle (3% gum acacia at the rate of 5ml/Kg/day) orally for 10 days.

Group II – Rats were administered 3% gum acacia for 10 days and on 8$^{th}$ day 2g/Kg paracetamol given orally.

Group III – Ethanolic extract of leaves of *Eugenia jambolana* was administered at a dose rate of 100 mg/kg orally in gum acacia for 10 days and paracetamol 2g/Kg given on 8$^{th}$ day.

Group IV – Ethanolic extract of leaves of *Eugenia jambolana* was administered at a dose rate of 200 mg/kg orally in gum acacia for 10 days and paracetamol 2g/Kg given on 8$^{th}$ day.

Group V – Rats were administered with silymarin 100mg/Kg/day in gum acacia for 10 days orally and on day 8, 2g/Kg paracetamol administered orally.

The blood was collected from all the animals on day 10 day and serum was separated and used for the estimation of biochemical parameters such as serum alanine aminotransferase, aspartate aminotransferase, albumin, total protein and bilirubin. Blood collected in anticoagulant Disodium salt of Ethylene Diamine Tetra Acetic acid (EDTA Sodium) at the rate of 1mg/ml (was used for studying the haematological parameters like Total leukocyte count ( TLC), Differential leukocyte count (DLC) and haemoglobin.

On day 10, the rats were sacrificed and liver was collected. These were used for the estimation of superoxide dismutase (SOD), catalase and also for conducting histopathological studies.

## 3.5 COLLECTION OF BIOLOGICAL SAMPLES

### 3.5.1 Collection of blood and separation of serum

Blood was collected from the retro orbital plexus under mild ether anaesthesia with heparinized capillary tubes, into sterile centrifuge tubes without adding any anticoagulant. It was kept at refrigeration temperature for half an hour, taken out and kept at room temperature for another half an hour. It was then centrifuged at 3200 rpm for 10 minutes and the clear serum obtained was pipetted out.

### 3.5.2 Liver

The animals were euthanized and dissected upon and the liver was collected. It was washed in running tap water to remove the blood clots and kept in chilled 0.9 percent sodium chloride.

## 3.6 OBSERVATIONS

### 3.6.1 Antioxidant enzymes

#### *3.6.1.1 Estimation of Superoxide dismutase*

Superoxide dismutase was estimated according to the procedure followed by Minami and Yoshikawa (1979).

**a. Reagents**

1. Tris cacodylic acid buffer (50mM, pH 8.2)

Tris cacodylic acid 50mM

Diethylene triamine penta acetic acid 1mM

Nitroblue tetrazolium 0.1mM

Triton X 100 0.001 percent

All the reagents were mixed in equal quantities and the pH was adjusted to 8.2 using 0.1N sodium hydroxide.

2. Sodium chloride 0.9 percent

3. Pyrogallol 0.2mM

**b. Procedure**

1. Freshly excised liver was homogenized with 10 volumes of 0.9 percent sodium chloride followed by centrifugation at 400 rpm for 10 minutes at $4^0$C to harvest the supernatant.
2. The assay mixture in a total volume of 3ml consisted of 1.4ml of 50mM tris cacodylic acid buffer, 1.4ml of 0.2mM pyrogallol and 0.2ml of enzyme preparation.
3. Blank contained distilled water instead of enzyme preparation
4. The absorbance due to autooxidation of pyrogallol was read at 420nm using 'Genesys' spectrophotometer.
5. One unit of SOD activity was the amount of enzyme which inhibited pyrogallol autooxidation by 50% under experimental conditions.
6. The values were expressed in units/mg of protein after quantifying the protein content of supernatant by method of Lowry *et al.* (1951).

### *3.6.1.2 Estimation of Catalase*

Catalase was estimated by the procedure followed by Cohen *et al.* (1970).

**a. Reagents**

1. Phosphate buffer-Hydrogen peroxide solution (10mM)

   Phosphate buffer – 0.05M, pH 7.0

   0.2 M sodium dihydrogen phosphate 39 ml

   0.2 M disodium hydrogen phosphate 61 ml

Immediately before use 0.12ml of hydrogen peroxide was added to 100ml buffer.

**b. Procedure**

1. 3 ml of the phosphate buffer-hydrogen peroxide solution was taken in test tubes.
2. Blank contained distilled water instead of hydrogen peroxide solution.
3. Samples prepared in sodium chloride (as described in case of superoxide dismutase) were added to both and the absorbance was read at 240nm at the $20^{th}$ second of addition of sample using 'Genesys' spectrophotometer.
4. The time required for the initial absorbance to decrease by 0.05 units was noted.
5. The catalase activity in units/assay mixture was calculated by using the formula $\log E_1/E_2 \times 2300/6.93 \times 1/\Delta t$.

   $E_1$- Initial absorbance

   $E_2$- Absorbance after decrease by 0.05 units

Δt- Time taken for the decrease in absorbance by 0.05 units (in seconds).

## 3.7 ESTIMATION OF SERUM PARAMETERS

The serum parameters were estimated colourimetrically in semi automatic blood analyzer (Microlab 200) by using the analytical kits supplied by Agappe Diagnostics Pvt. Ltd, Ernakulam, Kerala, India.

### 3.7.1 Alanine aminotransferase (ALT)

UV Kinetic test (Reitman and Frankel, 1957)

Principle

Serum ALT catalyses the transfer of aminogroup from L- alanine to L-oxoglutarate with formation of pyruvate and L- glutamate. The pyruvate so formed is allowed to react with NADH to produce L- lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDH in the presence of NADH. The oxidation of NADH is measured as the decrease in the absorbance of NADH at 340 nm, which is proportional to ALT activity.

Procedure:

Pipetted out 100µl of sample into 1000 µl working reagent, mixed well and measured the absorbance at 340 nm in an auto analyzer.

### 3.7.2 Aspartate aminotransferase (AST)

UV Kinetic test (Reitman and Frankel, 1957)

Aspartate aminotransferase catalyses transfer of amino group from L aspartate to 2- oxoglutarate forming oxaloacetate and L glutamate. The rate of this reaction is

monitored by an indicator reaction coupled with malate Dehydrogenase in the presence of NADH. The oxidation of NADH at 240 nm, which is proportional to AST activity.

Procedure

Mixed well 10 µl of sample and 1000 µl of working reagent and measured the absorbance at 340 nm in an auto analyzer.

### 3.7.3 Estimation of serum Total Protein

Biuret method (Gornall *et al.*, 1949)

Principle

The peptide bonds of protein react with copper ions in alkaline solution to form a blue- violet complex (so called biuret reaction). The complex formed is proportional to the protein concentration and is measured at 540 nm.

Procedure

Sample, standard and blank were prepared as follows

| | Sample | Standard | Blank |
|---|---|---|---|
| Serum | 10µl | | |
| Standard | | 10µl | |
| Reaction solution | 1000µl | 1000µl | 1000µl |

Mixed the solutions and incubated for ten minutes at 37 $^{0}$C. Read the absorbance of standard and sample against blank at 540 nm

Serum total protein (g/dl) = Absorbance of sample/ Absorbance of standard X 6

**3.7.4 Estimation of serum albumin** (Bromocresol Green Dye Method ,Doumas, 1971)

Principle

Albumin forms a blue green complex with bromocresol green at slightly acidic pH, which is measured photometrically.

Sample, standard and blank were prepared as follows

| | Sample | Standard | Blank |
|---|---|---|---|
| Serum | 10µl | | |
| Standard | | 10µl | |
| Reaction solution | 1000µl | 1000µl | 1000µl |

Mixed the solutions and incubated for ten minutes at 37 $^{0}$C. Read the absorbance of standard and sample against blank at 540 nm

Serum total protein (g/dl) = Absorbance of sample/ Absorbance of standard X 3

**3.7. 5 Estimation of bilirubin** (Modified DMSO method)

Principle

Sulfanilic acid reacts with sodium nitrite in the presence of diazotized sulfanilic acid to form azobilirubin. In the presence of dimethyl sulfoxide, only the direct bilirubin reacts to give azobilirubin.

Procedure

Sample, blank and test were prepared as follows

| | Sample blank | Test |
|---|---|---|
| Total bilirubin reagent | 1000µl | 1000µl |
| Activator Total | - | 20µl |
| Serum | 50µl | 50µl |

Mixed well and incubated for exactly 5 minutes. Measured the absorbance of the sample against respective sample blank at 546 nm.

Total bilirubin concentration in mg/dl= OD of sample (T) - OD of sample blank (T) X 20.5 (multiplication factor)

3.8 HAEMATOLOGICAL PARAMETERS

**3.8.1 Haemoglobin concentration**

Haemoglobin was estimated by acid Haematin method (Benjamin. 1985).

**3.8.2 Total leukocyte count**

Leucocytes were counted by standard dilution technique using Thomas fluid. Counting of leucocytes was done in the zone for leucocytes in the haemocytometer placed under low power of microscope (Benjamin, 1985).

### 3.8.3 Differential leukocyte count

Blood smears were prepared from freshly drawn blood without anticoagulant by using slide technique. After staining with Wrights stain counting was done under oil immersion (Benjamin, 1985).

## 3.9 GROSS AND HISTOPATHOLOGICAL EXAMINATION OF LIVER

The gross and histopathological lesions in liver were studied by collecting representative samples of liver obtained from the dissected animals on the $10^{th}$ day.

### 3.9.1 Gross lesions

The gross lesions in liver of treated groups were compared with control groups.

### 3.9.2 Histopathological examination

After sacrificing the animals on the $10^{th}$ day, liver was taken for histopathological examination to asses the hepatotoxicity. Three mm thick, pieces of liver were selected randomly from both control and experimental groups of rats and were fixed in 10 percent formalin. They were then processed and paraffin embedded as described by Sheehan and Hrapchak, 1980. The sections were stained with haematoxylin and eosin as per the technique followed by Bancroft and Cook, 1984. The sections were examined in detail under light microscope.

## 3.10 STATISTICAL ANALYSIS OF DATA

Results were analyzed by using analysis of variance (ANOVA) technique for comparison between groups and paired t test (Snedecor and Cochran, 1985). Results were expressed as mean ± standard error.

# *Results*

# 4. RESULTS

## 4.1 PHYTOCHEMICAL SCREENING

### 4.1.1 Steroids

No red colour and red ring were obtained in the Salkowski test and Lieberman Burchadt test respectively. Thus it could be concluded that steroids were not present in the ethanolic extract of *Eugenia jambolana.*

### 4.1.2 Alkaloids

No creamy white precipitate in Mayer's test and no characteristic yellow coloured precipitate with Hager's test were obtained with the extract. With Wagner's reagent, no characteristic reddish brown precipitate was obtained. Dragendroffs test also gave a negative result. So no detectable levels of alkaloids were present in the ethanolic extract of *Eugenia jambolana.*

### 4.1.3 Phenolic compounds

The extract mixed with 10 percent ferric chloride produced dark blue colour, which indicated the presence of phenolic compounds in *Eugenia jambolana* alcoholic extract

### 4.1.4 Tannins

Brownish green colour was obtained in ferric chloride test and white precipitate in gelatin test. It revealed the presence of tannins in the ethanolic extract of *Eugenia jambolana.*

### 4.1.5 Flavonoids

A green colour in the ferric chloride test and a characteristic yellow coloured precipitate in lead acetate test indicated the presence of flavonoids in the extract.

### 4.1.6 Glycosides

A red colour was obtained in the Benedict's test indicated the presence of glycosides in the sample. A yellow colour was obtained by mixing the extracts with sodium hydroxide reagent, which also indicated the presence of glycosides.

### 4.1.7 Diterpenes

Diterpenes were detected in *Eugenia jambolana* alcoholic extract as indicated by the green colour when mixed with copper sulphate solution.

### 4.1.8 Triterpenes

For *Eugenia jambolana* alcoholic extract, lower layer turned to yellow on standing as per Salkowski test, and by Leiberman Burchadt's test, a deep ring appeared at the junction of the two layers. These results indicated the presence of triterpenes in the extract.

### 4.1.9 Saponins

In the foam test, foam was not persisted for 10 minutes, which indicated that no saponins were present in the extract.

### 4.1.10 Nitrates

No characteristic bright blue colour was formed, which indicated that no nitrate was present in the fresh leaf of the plant.

### 4.1.11 Cyanides

Yellow picrate paper was not turned to red after incubation for 3 hours at 37° C. This indicated that no cyanide was present in the plant material.

The results obtained were summarized in the Table 1.

**Table 1. Results of phytochemical screening of *Eugenia jambolana* extract**

| Sl. No | Active principle | *Eugenia jambolana* |
|---|---|---|
| 1 | Steroids | Absent |
| 2 | Alkaloids | Absent |
| 3 | Tannins | Present |
| 4 | Flavonoids | Present |
| 5 | Glycosides | Present |
| 6 | Phenolic compounds | Present |
| 7 | Diterpenes | Present |
| 8 | Triterpenes | Present |
| 9 | Saponins | Absent |
| 10 | Nitrate | Absent |
| 11 | Cyanide | Absent |

## 4.2. PHYSIOLOGICAL PARAMETERS

### 4.2.1. Body weights

The individual and mean body weights of rats (group I, group II, group III, group IV and group V) were recorded on the day 0 and 10 of the experiment and are presented in the Table 2. The body weight recorded on the day 0 of groups I to V were 173.75 ± 4.60, 180.00 ± 4.62, 182.50 ± 4.90, 181.25 ± 2.95 and 178.75 ± 5.15 g respectively. After treatment on the $10^{th}$ day, the mean body weights recorded were 179.37 ± 3.71, 176.25 ± 5.40, 182.50 ± 4.90, 185.00 ± 3.13 and 182.5 ± 4.62 g respectively. A reduction in body weight was shown by group II animals and no change was shown by group III animals. A gradual increase in weight noticed was in the groups I, IV and V.

## 4.3 BIOCHEMICAL PARAMETERS

### 4.3.1 Antioxidant enzymes

#### *4.3.1.1 Superoxide dismutase*

The values obtained were presented in the Table 3 and Figure 2. The mean normal superoxide dismutase (SOD) level was found to be 49.26 ± 2.19 units/ mg protein. Out of five groups the SOD levels were lowest (22.28 ± 1.50) for group II animals (paracetamol treated groups). A significant increase in the level of SOD was noted in both the treatment groups when compared with paracetamol treated group (Group II). The SOD levels following treatment with ethanolic extract of *Eugenia jambolana* at doses 100mg/ Kg (Group III) and 200 mg/Kg (Group IV) were 46.11 ± 2.07 and 49.45 ± 1.67 units/mg protein respectively. The SOD level of silymarin treated animals (Group V) was 49.52 ± 1.25 units/ mg protein. There was no significant difference between Group III, IV and Group V animals.

**Table 2. Effect of treatment on body weights (g) of rats (n=8)**

| Animal No. | Group I | | Group II | | Group III | | Group IV | | Group V | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | $10^{th}$ day | Day 0 | $10^{th}$ day | Day 0 | $10^{th}$ day | Day 0 | $10^{th}$ day | Day 0 | $10^{th}$ day |
| 1 | 180 | 188 | 160 | 150 | 200 | 200 | 180 | 180 | 170 | 180 |
| 2 | 160 | 170 | 180 | 180 | 200 | 200 | 200 | 200 | 170 | 175 |
| 3 | 180 | 185 | 170 | 165 | 180 | 180 | 180 | 185 | 180 | 180 |
| 4 | 200 | 200 | 200 | 200 | 190 | 190 | 180 | 185 | 170 | 180 |
| 5 | 160 | 170 | 190 | 190 | 170 | 170 | 170 | 170 | 160 | 160 |
| 6 | 170 | 175 | 190 | 180 | 180 | 180 | 180 | 180 | 200 | 200 |
| 7 | 170 | 180 | 180 | 175 | 160 | 160 | 180 | 190 | 180 | 185 |
| 8 | 170 | 170 | 170 | 170 | 180 | 180 | 180 | 190 | 200 | 200 |
| Mean | 173.75 | 179.37 | 180.00 | 176.25 | 182.50 | 182.50 | 181.25 | 185.00 | 178.75 | 182.50 |
| SE | 4.60 | 3.71 | 4.62 | 5.40 | 4.90 | 4.90 | 2.95 | 3.13 | 5.15 | 4.62 |

*4.3.1.2 Catalase*

The results were shown in Table 4 and Figure 3. Catalase level in normal animals was found as 67.65 ± 3.17 units/assay mixture. A reduction in the catalase levels was noted in paracetamol treated groups when compared with the normal animals. Here the value is 42.60 ± 2.37. After 10 days of experiment the catalase levels in Group III, IV and V were found to be 66.17 ± 2.25, 74.60 ± 2.68 and 75.58 ± 2.24 units/ assay mixture respectively. The maximum value was noticed in the silymarin treated group V animals (75.58 ± 2.24). The catalase levels in Group IV and V were comparable.

## 4.4 SERUM PARAMETERS

### 4.4.1 Alanine amino transferase (ALT)

The results obtained were presented on the Tables 5 and 6 and Figure 4. The serum ALT levels before treatment were 54.12 ± 2.45, 56.25 ± 3.91, 55.62 ± 2.16, 54.75 ± 3.09 and 57.00 ± 3.13 U/L, respectively in groups I to V. The highest level of ALT was noticed in the paracetamol treated group, where the value increased from 56.25 ± 3.91 to 139. 62 ± 5.17 U/L. After ten days of experiment the level of ALT was 54.37 ± 2.47 in normal animals and in the extract treated animals the values became 118.12 ± 4.92 and 97.37 ± 4.70 U/L at 100 and 200 mg/Kg respectively. In the silymarin treated groups the values were near to the normal animals (62.00 ± 3.10 U/L). Group I and Group V animals did not differ significantly ($P<0.05$).

### 4.4.2 Aspartate amino transferase (AST)

The results obtained are presented in the Tables 7 and 8 and in Figure 5. The AST levels before treatment were 151.75 ± 7.41, 143.25 ± 7.03, 136.00 ± 6.07, 149.00 ± 5.06 and 147.37 ± 6.04 U/L respectively in group I, II, III, IV and V animals. After treatment, the value was highest in the paracetamol treated animals. Here the value increased to 274.62 ± 8.12 from 143.25 ± 7.03. After treatment the

values were 151.12 ± 7.2, 190.00 ± 6.37, 160.12 ± 4.95 and 147.62± 6.30 U/L respectively in groups I, III, IV and V animals. Group I, IV and V do not differ significantly ($P<0.05$).

### 4.4.3 Serum total protein

The values of serum total protein obtained before and after treatment were presented on Table 9 and 10 and Figure 6. The means on the day 0 were 6.76 ± 0.11, 6.65 ± 0.13, 6.58 ± 0.11, 6.32 ± 0.15 and 6.30 ± 0.12 g/dl respectively, for groups I to V. After the treatment with ethanolic extract of *Eugenia jambolana* at the dose of 100 and 200 mg/ Kg, the level changed to 6.58 ± 0.13 and 6.45 ± 0.11 g/dl respectively. The value of group I animals were same before and after the treatment. The total protein level of Group II animals decreased to 6.41 ± 0.17 from 6.65 ± 0.13 g/dl. The values of Group I and V were 6.76 ± 0 .08 and 6.46 ± 0.10 respectively.

### 4.4.4 Serum albumin

The data obtained before and after treatment were shown in Tables 11 and 12 and Figure 7. On the day 0, the mean serum albumin levels of Groups I to V were 2.38 ± 0.09, 2.41 ± 0.10, 2.71 ± 0.07, 2.37 ± 0.12 and 2.5 ± 0.05 g/dl respectively. After the treatment with *Eugenia jambolana* extract at the 100 and 200 mg/ Kg doses, the values were changed to 2.78 ± 0.05 and 2.51 ± 0.07 respectively in Group III and IV animals. The level for Group I and V were 2.33 ± 0.09 and 2.47 ± 0.06 g/dl respectively. A reduction in value was noticed in the paracetamol treated group. Here the value was decreased to 2.02 ± 0.05 g/dl. There was no significant difference between the groups I, IV and V.

### 4.4.5 Serum total bilirubin

The results obtained before and after treatment were shown in Tables 13 and 14 and Figure 8. The serum total bilirubin values before the treatment were 0.34 ± 0 .01, 0.35 ± 0.01, 0.35 ± 0.03 and 0.32 ± 0.02 mg/dl respectively for the groups I to V.

**Table 3. Effect of *Eugenia jambolana* on superoxide dismutase level (units/mg protein) in paracetamol induced hepatotoxicity in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 42.7 | 19.6 | 40.3 | 47.4 | 51.4 |
| 2 | 55.2 | 23.4 | 42.4 | 49.5 | 42.8 |
| 3 | 48.3 | 25.8 | 51.7 | 39.8 | 53.7 |
| 4 | 57.4 | 17.4 | 54.3 | 54.2 | 47.4 |
| 5 | 48.9 | 19.1 | 41.3 | 55.3 | 49.2 |
| 6 | 54.3 | 18.7 | 49.2 | 50.7 | 53.1 |
| 7 | 39.4 | 24.8 | 39.4 | 48.5 | 50.7 |
| 8 | 47.9 | 29.5 | 50.3 | 50.2 | 47.9 |
| Mean | $49.26^{a}$ | $22.28^{b}$ | $46.11^{a}$ | $49.45^{a}$ | $49.52^{a}$ |
| SE | 2.19 | 1.50 | 2.07 | 1.67 | 1.25 |

Means bearing the same superscripts do not differ significantly at $P<0.05$

**Table 4. Effect of *Eugenia jambolana* on catalase level (units/assay mixture) in paracetamol induced hepatotoxicity in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 75.2 | 40.7 | 65.8 | 78.5 | 82.7 |
| 2 | 59.3 | 35.8 | 54.4 | 79.9 | 81.7 |
| 3 | 64.3 | 32.3 | 71.7 | 81.7 | 69.9 |
| 4 | 71.2 | 44.2 | 66.4 | 69.5 | 74.3 |
| 5 | 65.8 | 47.8 | 72.1 | 67.8 | 70.6 |
| 6 | 54.7 | 39.4 | 66.9 | 61.8 | 74.3 |
| 7 | 83.2 | 49.7 | 72.4 | 74.4 | 83.8 |
| 8 | 67.5 | 50.9 | 59.7 | 83.2 | 67.4 |
| Mean | $67.65^{ab}$ | $42.60^{c}$ | $66.17^{a}$ | $74.60^{bd}$ | $75.58^{d}$ |
| SE | 3.17 | 2.37 | 2.25 | 2.68 | 2.24 |

Means bearing the same superscripts do not differ significantly at $P<0.05$

**Table 5. Serum ALT level (U/L) before treatment in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 54 | 53 | 47 | 53 | 61 |
| 2 | 45 | 47 | 54 | 62 | 55 |
| 3 | 65 | 49 | 63 | 39 | 64 |
| 4 | 62 | 39 | 48 | 49 | 57 |
| 5 | 57 | 68 | 53 | 64 | 52 |
| 6 | 48 | 59 | 60 | 57 | 48 |
| 7 | 49 | 70 | 62 | 50 | 46 |
| 8 | 53 | 65 | 58 | 64 | 73 |
| Mean | $54.12^{a}$ | $56.25^{d}$ | $55.62^{c}$ | $54.75^{b}$ | $57.00^{a}$ |
| SE | 2.45 | 3.91 | 2.16 | 3.09 | 3.13 |

Means bearing the same superscripts do not differ significantly at $P<0.05$

**Table 6. Effect of *Eugenia jambolana* on ALT (U/L) in paracetamol induced hepatotoxicity in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 56 | 129 | 118 | 90 | 72 |
| 2 | 44 | 138 | 105 | 87 | 63 |
| 3 | 64 | 149 | 99 | 103 | 69 |
| 4 | 62 | 128 | 109 | 117 | 70 |
| 5 | 58 | 170 | 118 | 105 | 64 |
| 6 | 47 | 132 | 132 | 89 | 53 |
| 7 | 51 | 127 | 123 | 78 | 47 |
| 8 | 53 | 144 | 141 | 110 | 58 |
| Mean | $54.37^{a}$ | $139.62^{b}$ | $118.12^{c}$ | $97.37^{d}$ | $62.00^{a}$ |
| SE | 2.47 | 5.17 | 4.92 | 4.70 | 3.10 |

Means bearing the same superscripts do not differ significantly at $P<0.05$

**Table 7. Serum AST level (U/L) before treatment in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 128 | 130 | 134 | 162 | 158 |
| 2 | 154 | 164 | 125 | 127 | 133 |
| 3 | 149 | 159 | 107 | 153 | 156 |
| 4 | 175 | 137 | 152 | 137 | 127 |
| 5 | 188 | 129 | 138 | 159 | 132 |
| 6 | 152 | 161 | 164 | 133 | 142 |
| 7 | 137 | 157 | 139 | 159 | 178 |
| 8 | 131 | 109 | 129 | 162 | 153 |
| Mean | 151.75 | 143.25 | 136.00 | 149.00 | 147.37 |
| SE | 7.41 | 7.03 | 6.07 | 5.06 | 6.04 |

**Table 8.Effect of *Eugenia jambolana* on AST (U/L) in paracetamol induced hepatotoxicity in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 133 | 240 | 188 | 184 | 165 |
| 2 | 145 | 282 | 197 | 164 | 140 |
| 3 | 152 | 299 | 217 | 165 | 153 |
| 4 | 173 | 281 | 175 | 144 | 120 |
| 5 | 187 | 240 | 163 | 168 | 133 |
| 6 | 155 | 297 | 203 | 140 | 140 |
| 7 | 136 | 285 | 175 | 162 | 175 |
| 8 | 128 | 273 | 202 | 154 | 155 |
| Mean | $151.12^{a}$ | $274.62^{b}$ | $190.00^{c}$ | $160.12^{a}$ | $147.62^{a}$ |
| SE | 7.20 | 8.12 | 6.37 | 4.95 | 6.30 |

Means bearing the same superscripts do not differ significantly at $P<0.05$

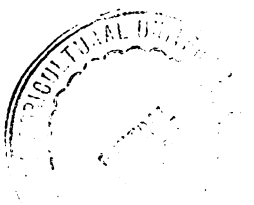

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**Table 9. Serum Total protein level (g/dl) before treatment in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 6.7 | 6.3 | 6.3 | 7.0 | 6.9 |
| 2 | 6.5 | 6.1 | 6.1 | 6.2 | 6.4 |
| 3 | 7.0 | 6.5 | 6.5 | 5.8 | 6.3 |
| 4 | 6.4 | 7.0 | 7.1 | 5.9 | 5.9 |
| 5 | 6.3 | 7.2 | 6.5 | 6.3 | 6.2 |
| 6 | 6.9 | 6.5 | 6.7 | 6.9 | 6.7 |
| 7 | 7.1 | 6.7 | 7.0 | 6.1 | 6.1 |
| 8 | 7.2 | 6.9 | 6.5 | 6.4 | 5.9 |
| Mean | 6.76 | 6.65 | 6.58 | 6.32 | 6.30 |
| SE | 0.11 | 0.13 | 0.11 | 0.15 | 0.12 |

**Table 10.Effect of *Eugenia jambolana* on Total protein level in paracetamol induced hepatotoxicity in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 6.8 | 5.9 | 6.4 | 6.9 | 6.8 |
| 2 | 6.4 | 6.0 | 5.9 | 6.4 | 6.7 |
| 3 | 6.9 | 6.1 | 6.4 | 6.1 | 6.4 |
| 4 | 6.8 | 6.2 | 7.0 | 6.0 | 6.2 |
| 5 | 6.5 | 7.1 | 6.8 | 6.2 | 6.7 |
| 6 | 6.7 | 7.0 | 6.4 | 6.8 | 6.2 |
| 7 | 6.9 | 6.9 | 6.9 | 6.5 | 6.0 |
| 8 | 7.1 | 6.1 | 6.9 | 6.7 | 6.7 |
| Mean | 6.76 | 6.41 | 6.58 | 6.45 | 6.46 |
| SE | 0.08 | 0.17 | 0.13 | 0.11 | 0.10 |

**Table 11. Serum albumin level (g/dl) before treatment in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 2.1 | 2.8 | 2.8 | 2.3 | 2.4 |
| 2 | 2.2 | 2.1 | 2.9 | 2.9 | 2.5 |
| 3 | 2.8 | 2.5 | 2.5 | 2.0 | 2.2 |
| 4 | 2.5 | 2.2 | 2.3 | 2.1 | 2.7 |
| 5 | 2.4 | 2.3 | 2.9 | 2.6 | 2.9 |
| 6 | 2.3 | 2.9 | 2.8 | 2.8 | 2.4 |
| 7 | 2.1 | 2.1 | 2.9 | 2.1 | 2.3 |
| 8 | 2.7 | 2.4 | 2.6 | 2.2 | 2.1 |
| Mean | 2.38 | 2.41 | 2.71 | 2.37 | 2.52 |
| SE | 0.09 | 0.10 | 0.07 | 0.12 | 0.05 |

**Table 12. Effect of *Eugenia jambolana* on serum albumin (g/dl) in paracetamol induced hepatotoxicity in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 2.2 | 1.9 | 2.9 | 2.4 | 2.5 |
| 2 | 2.0 | 2.0 | 2.7 | 2.8 | 2.4 |
| 3 | 2.5 | 2.3 | 2.7 | 2.4 | 2.3 |
| 4 | 2.5 | 2.1 | 2.5 | 2.3 | 2.6 |
| 5 | 2.4 | 2.2 | 2.7 | 2.8 | 2.8 |
| 6 | 2.2 | 2.0 | 2.5 | 2.7 | 2.6 |
| 7 | 2.1 | 1.9 | 2.9 | 2.3 | 2.4 |
| 8 | 2.8 | 1.8 | 3.0 | 2.4 | 2.2 |
| Mean | 2.33 [a] | 2.02 [b] | 2.78 [c] | 2.51 [a] | 2.47 [a] |
| SE | 0.09 | 0.5 | 0.05 | 0.07 | 0.06 |

Means bearing the same superscripts do not differ significantly at $P<0.05$

**Table 13. Serum total bilirubin (mg/dl) before treatment in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 0.4 | 0.3 | 0.4 | 0.3 | 0.3 |
| 2 | 0.3 | 0.4 | 0.3 | 0.4 | 0.4 |
| 3 | 0.3 | 0.3 | 0.3 | 0.2 | 0.4 |
| 4 | 0.4 | 0.4 | 0.4 | 0.3 | 0.4 |
| 5 | 0.3 | 0.4 | 0.3 | 0.4 | 0.3 |
| 6 | 0.3 | 0.3 | 0.4 | 0.5 | 0.2 |
| 7 | 0.3 | 0.3 | 0.4 | 0.4 | 0.3 |
| 8 | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 |
| Mean | 0.34 | 0.35 | 0.35 | 0.35 | 0.32 |
| SE | 0.01 | 0.01 | 0.01 | 0.03 | 0.02 |

**Table 14. Effect of *Eugenia jambolana* on serum total bilirubin (mg/dl) in paracetamol induced hepatotoxicity in rats (n=8)**

| Animal No | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| 1 | 0.4 | 0.8 | 0.4 | 0.4 | 0.4 |
| 2 | 0.4 | 1.1 | 0.4 | 0.7 | 0.3 |
| 3 | 0.3 | 1.0 | 0.5 | 0.4 | 0.4 |
| 4 | 0.4 | 1.3 | 0.5 | 0.3 | 0.3 |
| 5 | 0.3 | 1.2 | 0.4 | 0.3 | 0.2 |
| 6 | 0.3 | 0.8 | 0.3 | 0.4 | 0.3 |
| 7 | 0.3 | 1.1 | 0.4 | 0.3 | 0.4 |
| 8 | 0.4 | 0.9 | 0.5 | 0.4 | 0.3 |
| Mean | $0.35^{a}$ | $1.02^{b}$ | $0.42^{a}$ | $0.40^{a}$ | $0.34^{a}$ |
| SE | 0.01 | 0.06 | 0.02 | 0.04 | 0.02 |

Means bearing the same superscripts do not differ significantly at $P<0.05$

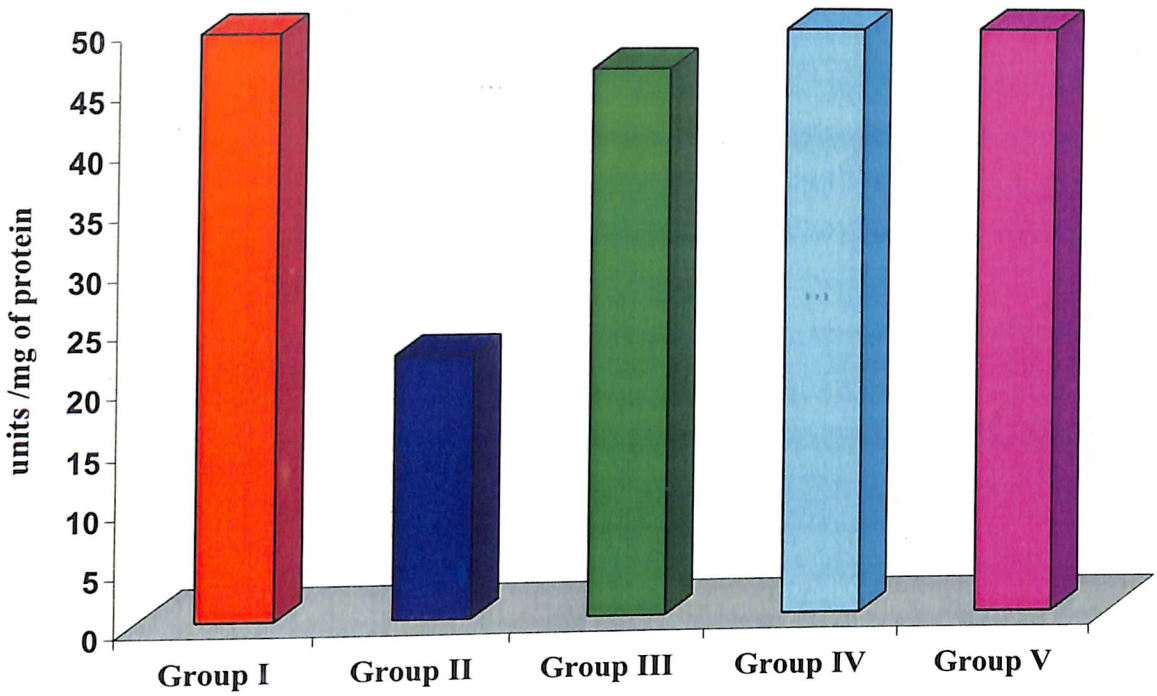



**Fig.2. Effect of treatment of SOD level (units/mg of protein)**

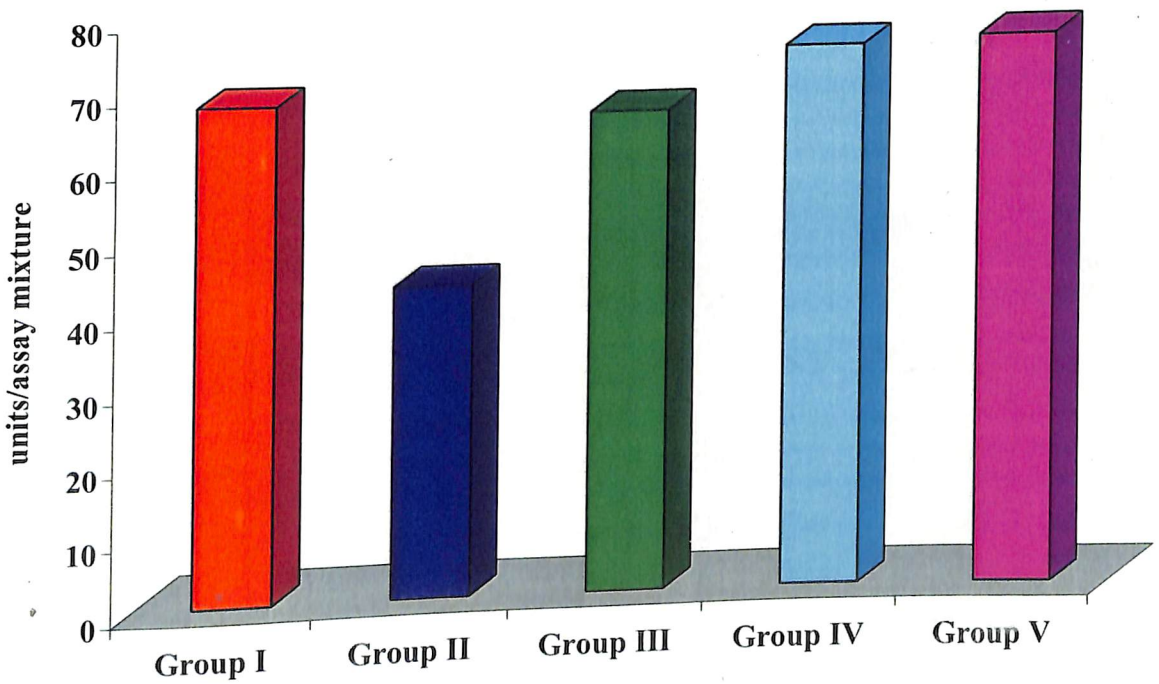
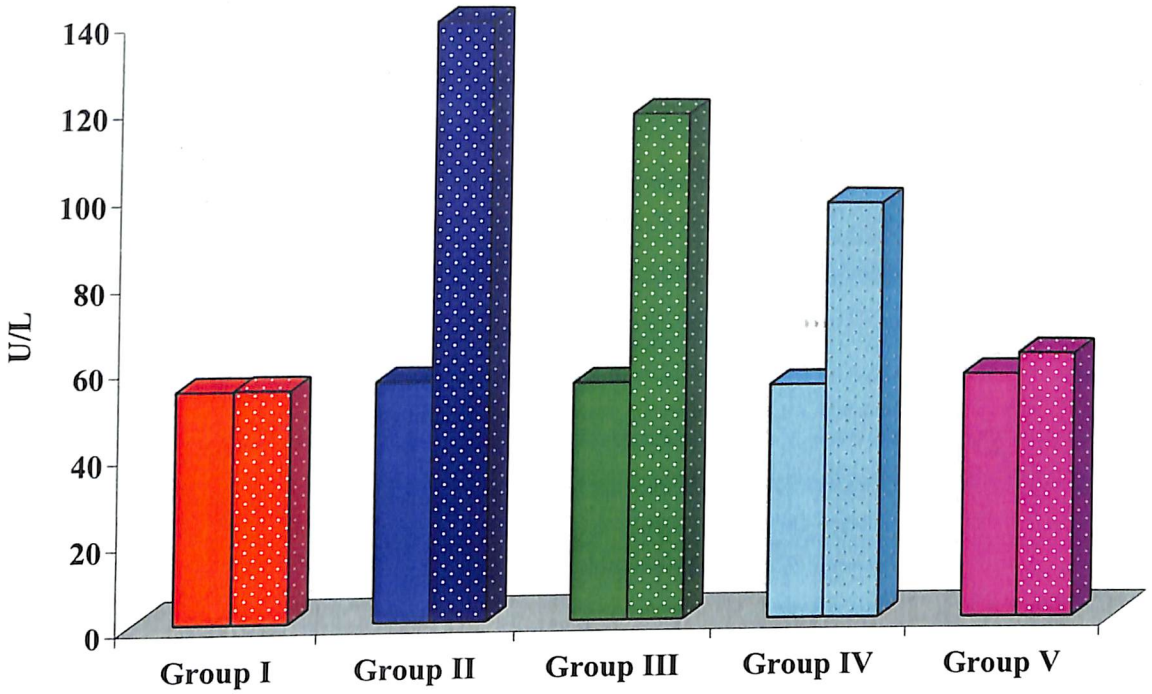
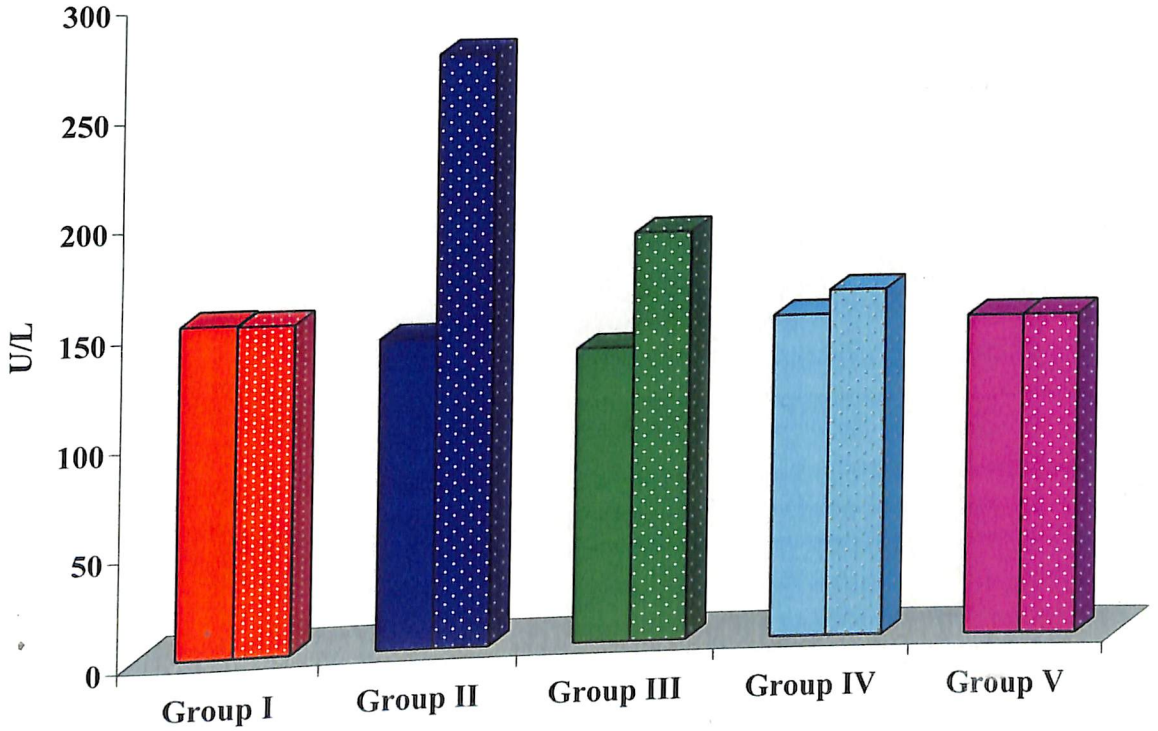



**Fig.3. Effect of treatment on catalase level (units/assay mixture)**



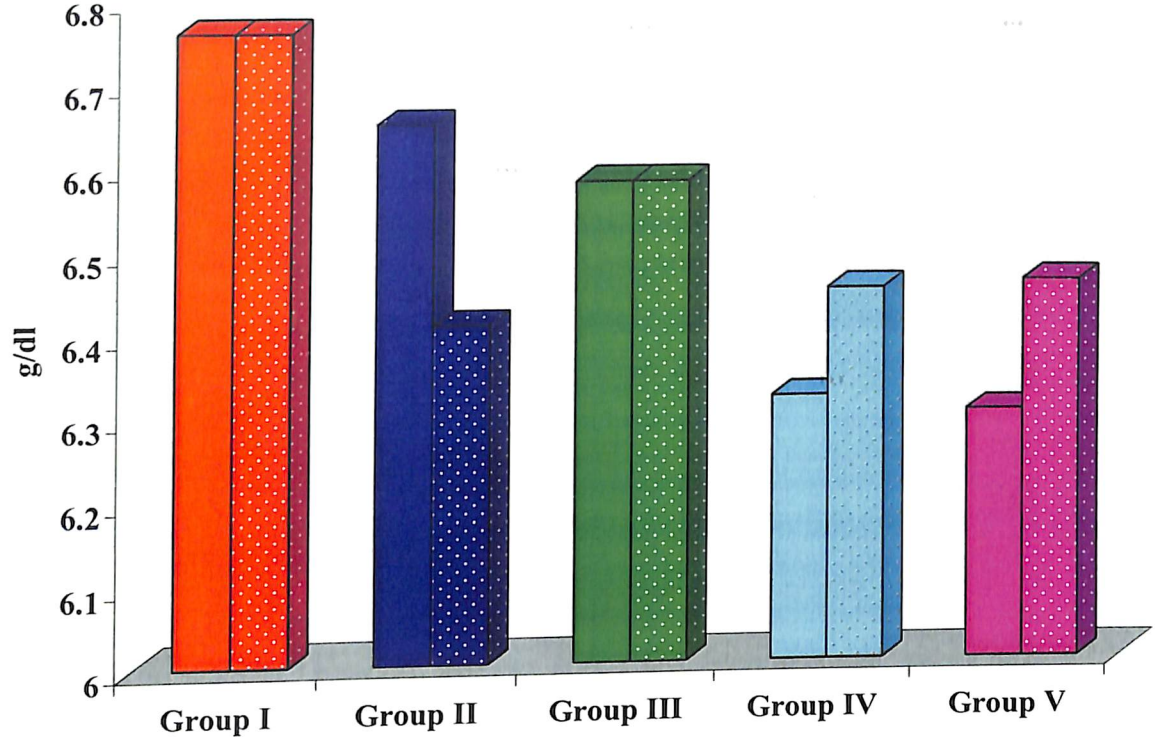

Dotted bars indicate levels after treatment

**Fig. 4. Effect of treatment on ALT level (U/L)**



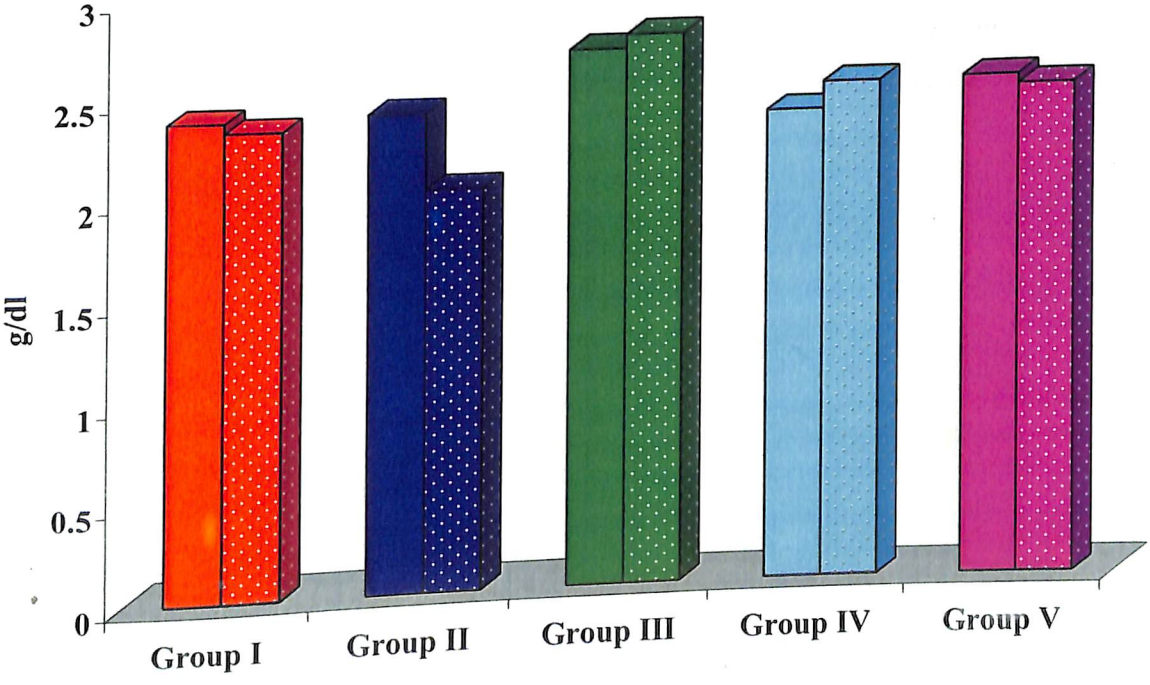

Dotted bars indicate levels after treatment

**Fig. 5. Effect of treatment on AST level (U/L)**



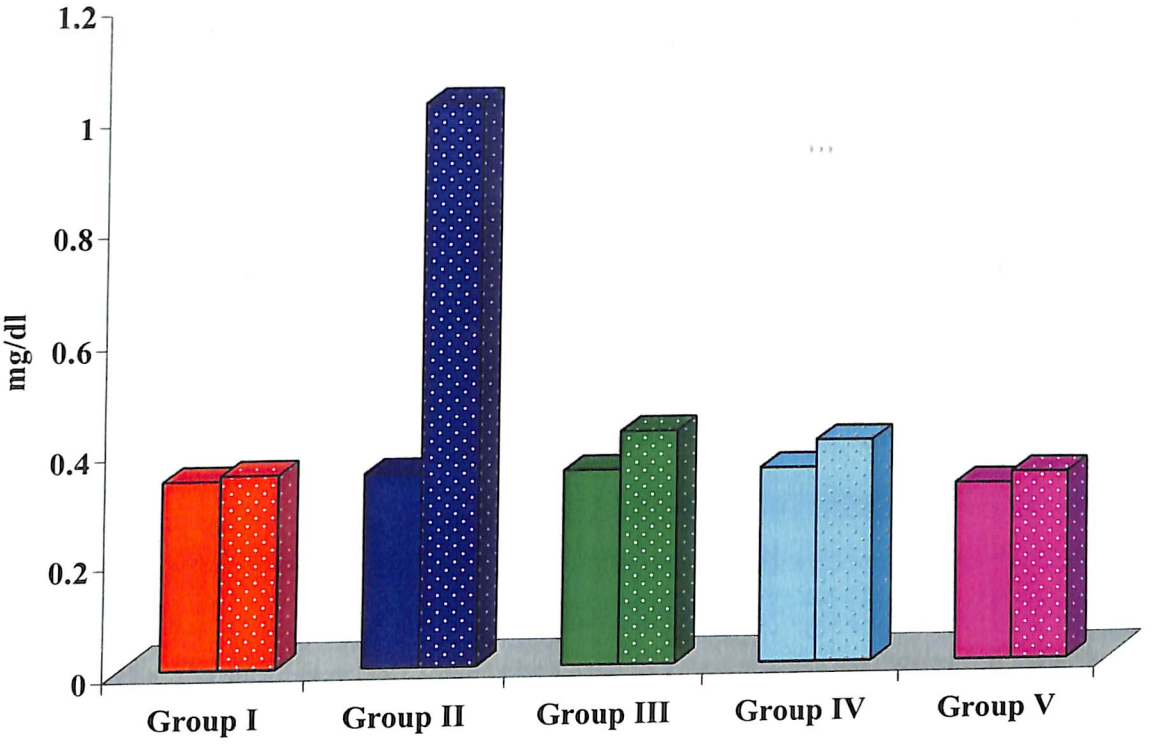

Dotted bars indicate levels after treatment

**Fig. 6. Effect of treatment on total protein level (g/dl)**




Dotted bars indicate levels after treatment

**Fig. 7. Effect of treatment on albumin level (g/dl)**




Dotted bars indicate levels after treatment

**Fig. 8. Effect of treatment on total bilirubin level (mg/dl)**

A high level of bilirubin 1.02 ± 0.06 mg/dl was noticed in the Group II animals. The level of total bilirubin in the other groups were 0.35 ± 0.01, 0.42 ± 0.02, 0.40 ± 0.04 and 0.34 ± 0.02 mg/dl for the groups I, III, 1V and V respectively. Groups I, III, IV and V did not differ significantly ($P<0.05$).

## 4.5 HAEMATOLOGICAL PARAMETERS

### 4.5.1 Haemoglobin concentration

The mean values were presented in the Tables 15 and 16. The haemoglobin concentration of group I, II, III, IV and Group V animals before the treatment is 10.00 ± 0.40, 10.50 ± 0.35, 10.81 ± 0 .29, 9.68 ± 0.33 and 10.75 ± 0.35 g/dl. After the experiment the concentrations were 10.12 ± 0.32, 10.31 ± 0.31, 10.81 ± 0.32, 9.75 ± 0.26 and 10.68 ± 0.40 g/dl respectively for the groups I to V. The values were in normal range on both the occasions in all the groups.

### 4.5.2 Total Leukocyte Count (TLC)

The values before and after treatment were presented in Tables 15 and 16. All the values were in the normal range before and after the treatment. On the day 0, the values were 11.43 ± 0.29, 11.70 ± 0.29, 11.42 ± 0.32, and 11.60 ± 0.35 and 11.90 ± 0.39 X $10^3/\mu l$ respectively. After the treatment the values were 11.58 ± 0.22, 11.13 ± 0.42, 11.46 ± 0.39, 11.67 ± 0.34 and 12.30 ± 0.35 X $10^3/\mu l$.

### 4.5.3 Differential Leukocyte Count (DLC)

#### *4.5.3.1 Lymphocytes*

The results obtained were in the Tables 15 and 16. Before treatment the values were 79.92 ± 0.54, 79.62 ± 0.48, 78.93 ± 0.46, 79.08 ± 0.35 and 79.07 ± 0.34 percent for the groups I to V. After the treatment with the plant extract the values were 78.76 ± 0.43 and 78.67 ± 0.46 percent respectively. In the group II animals the mean value was 79.17 ± 0.34 percent.

**Table 15. Haematological values before treatment in rats**

| | | Haemoglobin (g/dl) | Leucocyte count ($10^3/\mu l$) | Differential leucocyte count (%) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Lymphocyte | Neutrophil | Eosinophil | Monocyte | Basophil |
| Group I | Mean | 10.00 | 11.43 | 79.92 | 18.47 | 0.61 | 0.14 | 0.00 |
| | SE | 0.40 | 0.29 | 0.54 | 0.47 | 0.06 | 0.05 | 0.00 |
| Group II | Mean | 10.50 | 11.70 | 79.62 | 19.65 | 0.61 | 0.15 | 0.00 |
| | SE | 0.35 | 0.29 | 0.48 | 0.34 | 0.02 | 0.06 | 0.00 |
| Group III | Mean | 10.81 | 11.42 | 78.93 | 20.22 | 0.67 | 0.15 | 0.00 |
| | SE | 0.29 | 0.32 | 0.46 | 0.37 | 0.03 | 0.07 | 0.00 |
| Group IV | Mean | 9.68 | 11.60 | 79.08 | 19.63 | 0.66 | 0.12 | 0.00 |
| | SE | 0.33 | 0.35 | 0.35 | 0.36 | 0.05 | 0.09 | 0.00 |
| Group V | Mean | 10.75 | 11.90 | 79.07 | 19.91 | 0.67 | 0.14 | 0.00 |
| | SE | 0.35 | 0.39 | 0.34 | 0.40 | 0.03 | 0.06 | 0.00 |

**Table 16. Effect of *Eugenia jambolana* on haematological parameters in paracetamol induced hepatotoxicity in rats (n=8)**

| | | Haemoglobin (g/dl) | Leucocyte count ($10^3$/µl) | Differential leucocyte count (%) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Lymphocyte | Neutrophil | Eosinophil | Monocyte | Basophil |
| Group I | Mean | 10.12 | 11.58 | 69.25 | 18.51 | 0.66 | 0.14 | 0.00 |
| | SE | 0.32 | 0.22 | 9.89 | 0.40 | 0.05 | 0.04 | 0.00 |
| Group II | Mean | 10.31 | 11.13 | 79.17 | 19.65 | 0.63 | 0.15 | 0.00 |
| | SE | 0.31 | 0.42 | 0.34 | 0.32 | 0.02 | 0.04 | 0.00 |
| Group III | Mean | 10.81 | 11.46 | 78.76 | 19.86 | 0.63 | 0.15 | 0.00 |
| | SE | 0.32 | 0.39 | 0.43 | 0.29 | 0.03 | 0.05 | 0.00 |
| Group IV | Mean | 9.75 | 11.67 | 78.67 | 19.70 | 0.65 | 0.12 | 0.00 |
| | SE | 0.26 | 0.34 | 0.46 | 0.45 | 0.04 | 0.06 | 0.00 |
| Group V | Mean | 10.68 | 12.30 | 79.12 | 19.70 | 0.66 | 0.14 | 0.00 |
| | SE | 0.40 | 0.35 | 0.32 | 0.36 | 0.03 | 0.08 | 0.00 |

*4.5.3.2 Neutrophils*

The data were presented in the Tables 15 and 16. The Neutrophil count of the rats before treatment were 18.47 ± 0.47, 19.65 ± 0.34, 20.22 ± 0.37, 19.63 ± 0.36 and 19.91 ± 0.40 percent respectively for the groups I to V. After the experiment on the 10$^{th}$ day the values obtained were 18.47 ± 0.47, 19.65 ± 0.34, 20.22 ± 0.37, 19.63 ± 0.36 and 19.91 ± 0.40 percent respectively for the groups I to V.

*4.5.3.3 Eosinophils*

The results obtained were presented in Tables 15 and 16. The values were 0.61 ± 0.06, 0.61 ± 0.02, 0.67 ± 0.03, 0.66 ± 0.05 and 0.67 ± 0.03 percent for the groups I to V. After the treatment with the plant extract the values were recorded as 0.66 ± 0.05, 0.63 ± 0.02, 0.63 ± 0.03, 0.65 ± 0.04 and 0.66 ± 0.03 percent respectively.

*4.5.3.4 Monocytes*

The data was presented in the tables 15 and 16. The Monocyte count before treatment were 0.14 ± 0.05, 0.15 ± 0.06, 0.15 ± 0.07, 0.12 ± 0.09 and 0.14 ± 0.08 percent and after the treatment the values were 0.14 ± 0.04, 0.15 ± 0.04, 0.15 ± 0.05, 0.12 ± 0.06 and 0.14 ± 0.08 percent respectively for the groups I, II, III, IV and Group V.

*4.5.3.5 Basophils*

Basophils were not observed before and after the treatment.

4.6 GROSS AND HISTOPATHOLOGICAL EXAMINATION OF LIVER

Gross examination of liver from all the groups showed normal appearance and colour except the paracetamol treated group (Fig. 9). Paracetamol treated group showed marked greyish white areas of necrosis and diffuse petichae (Fig. 10).

Histopathological examination of the liver from the normal group revealed normal hepatic architecture (Fig. 11). In the paracetamol treated group, only ring of hepatocytes and there were extensive areas of coagulation necrosis and sinusoidal congestion (Fig. 12). In the 100mg/Kg extract treated group, liver showed diffuse necrotic areas involving only certain lobules (Fig. 13). The hepatocytes surrounding those areas were hypertrophic. A few mononuclear cells were seen in the necrotic area. Kupffer cell reaction was prominent. In the 200mg/Kg extract treated group necrosis involving only a very few hepatocytes in certain lobules and it showed almost normal hepatic architecture (Fig. 14). The hepatocytes seen around such lesions were hypertrophic and some of the hepatocytes have hyperchromatic nuclei. Silymarin treated group showed almost normal liver architecture (Fig. 15) except for some mild foci of coagulation.

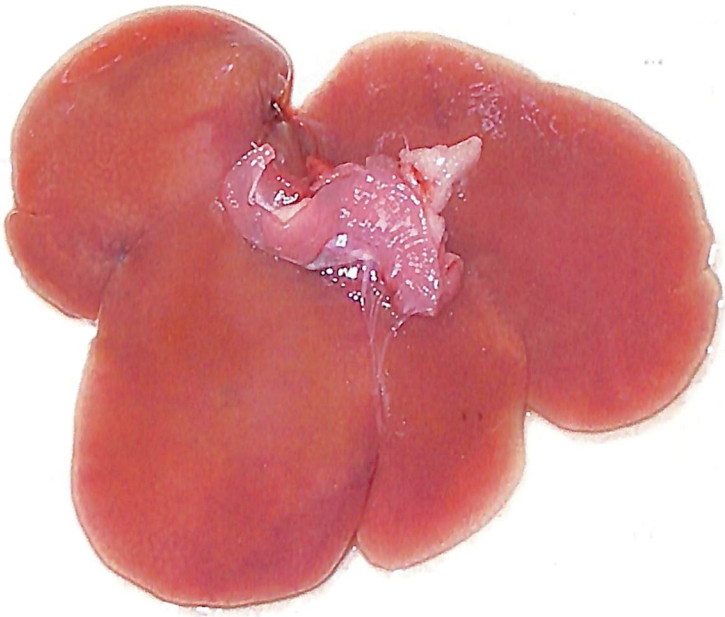


Fig. 9.Normal liver

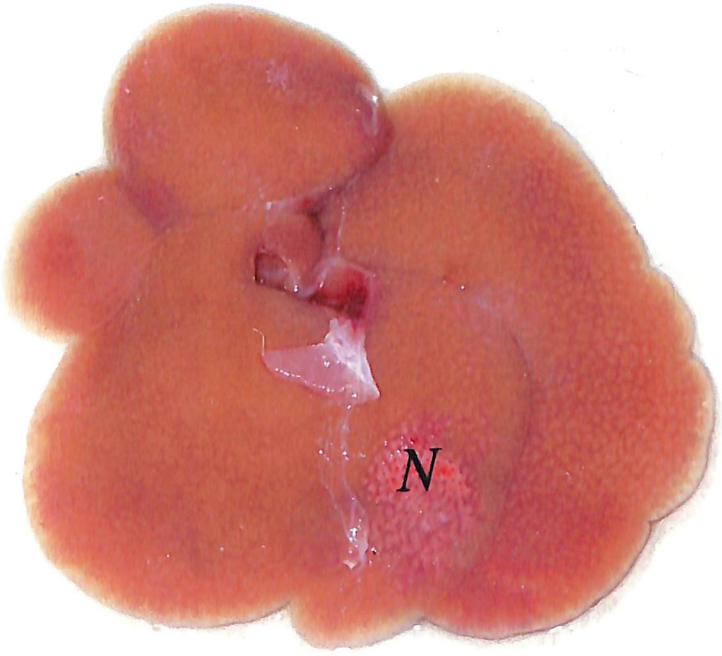



Fig. 10. Liver of paracetamol treated group showing areas of necrosis(N)

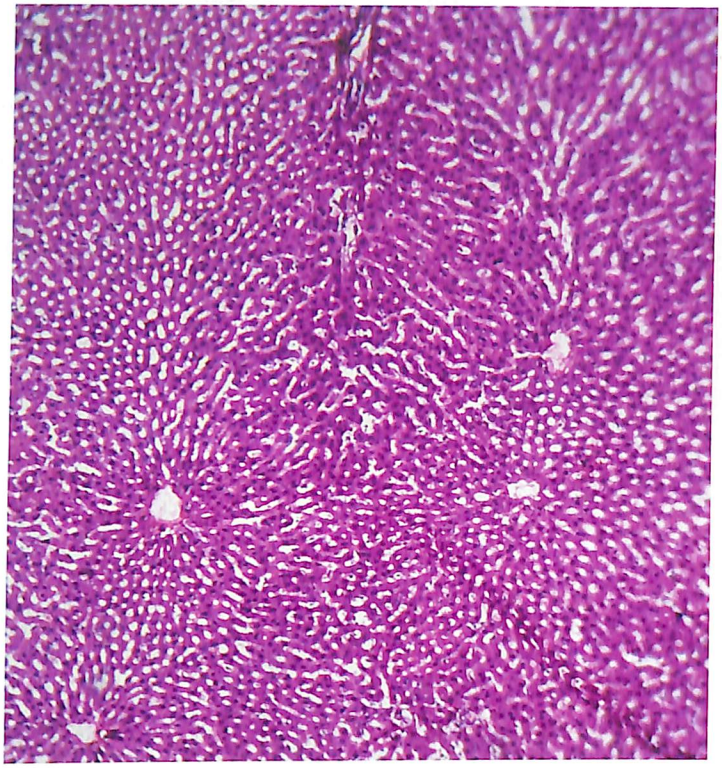


Fig. 11. Liver - healthy control
Normal arrangement of hepatocytes(H&Ex100)

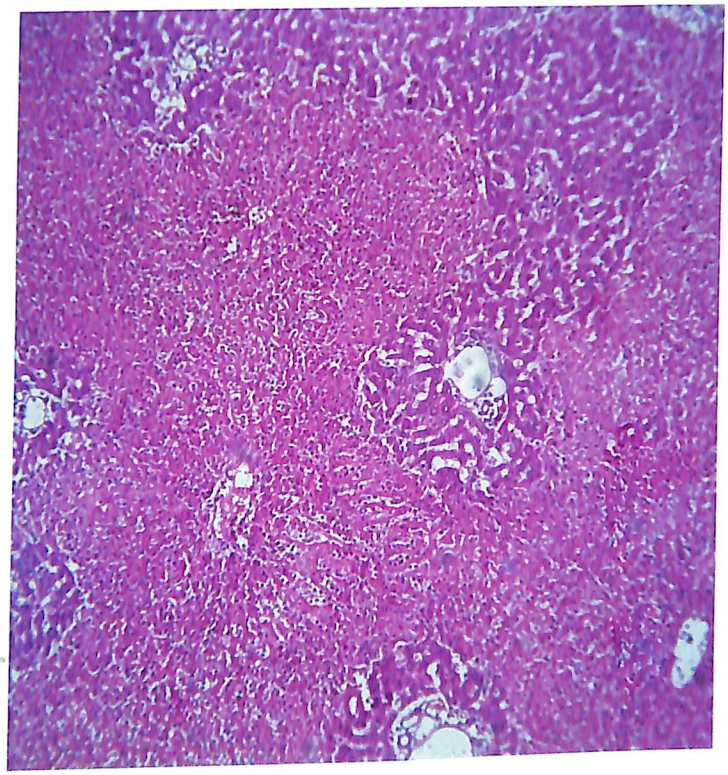


Fig. 12. Liver - paracetamol
treated group showing centrilobular
coagulation necrosis (H&Ex100)

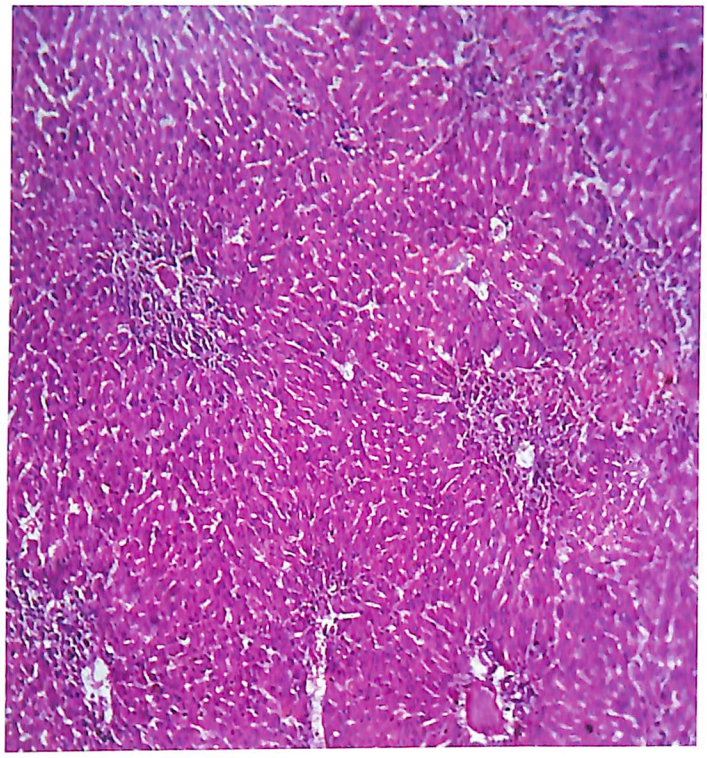


Fig. 13. Liver- *Eugenia jambolana* 100 mg/ Kg

Diffuse necrosis and pericentral inflammatory cells (H&Ex100)

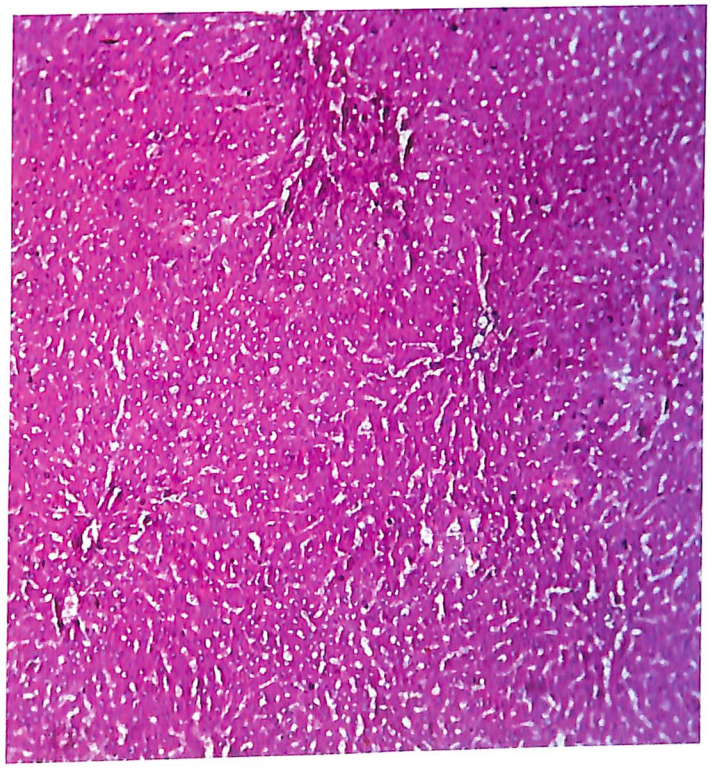


Fig. 14. Liver-*Eugenia jambolana* 200 mg/ Kg

Hepatic regeneration, normal hepatic architecture (H&Ex100)

# *Discussion*

# 5. DISCUSSION

The liver is the key organ regulating homeostasis in the body. It is involved in almost all the biochemical pathways related to growth, fight against diseases, nutrient supply and energy provision. The liver not only performs physiological functions but protects against the hazards of harmful drugs and chemicals also.

In spite of tremendous scientific advancement in the field of medicine in recent years, ailments due to liver disorders are on the rise. Liver diseases such as jaundice, cirrhosis and fatty liver are the very common hepatic disorders that account for a high death rate. Presently only a few hepatoprotective drugs and that too from natural sources are available for the treatment of liver disorders. Drugs like N- acetyl cysteine and silymarin were used to treat various liver disorders. Some vitamins like vitamin C, E and B complex were also used because of their antioxidant properties. Herbal medicines derived from plant extracts are increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. In India, mainly polyherbal formulations were used to treat liver disorders. There are herbal drugs like *Phyllanthus niruri,* which were used traditionally for the treatment of jaundice. There are various other herbal agents, but their effects are not scientifically validated. Hence the present study was undertaken to evaluate the hepatoprotective effect of ethanolic extract of *Eugenia jambolana* in paracetamol induced hepatotoxicity.

In the present study paracetamol is used to induce the hepatotoxicity. It is widely used as an analgesic drug with least gastrointestinal disturbances. The other hepatotoxic agents like $CCl_4$, antitubercular drugs, thioacetamide, galactosamide, ethanol and sodium oxalate can be used to induce hepatotoxicity experimentally. Most of the hepatotoxic agents cause liver damage mainly by lipid peroxidation and also by other oxidative damages. Paracetamol is the deethylated product of phenacetin and it can produce acute liver damage by the covalent binding of N- acetyl- *p*-Benzoquinone imine, an oxidation product of paracetamol to sulfhydryl groups of protein resulting in cell necrosis and thereby damage.

There were five groups of eight animals each group. Blood was collected prior to the experiment for the estimation of serum parameters and haematological parameters. Control group was administered with 3% gum acacia (vehicle) 5ml/Kg for 10 days. Groups III and IV administered with *Eugenia jambolana* leaf extract at the dose rate 100 and 200 mg/ Kg respectively and the group V administered with silymarin at the dose of 100mg/Kg/day in 3% gum acacia. Groups II, III, IV and V were administered with paracetamol on eighth day at the dose rate of 2g/Kg. Fourty eight hours after the administration of paracetamol, all the animals were sacrificed after blood collection and biochemical and haematological parameters were estimated and conducted histopathology of liver.

## 5.1 PHYTOCHEMICAL SCREENING

Phytochemical screening of the ethanolic extract of *Eugenia jambolana* revealed the presence of phenolic compounds, flavonoids, tannins, glycosides, diterpenes and triterpenes.

Ethyl acetate extract of *Eugenia jambolana* leaves was found to contain a complicated mixture of polyphenols, specially flavonols, glycosides and some phenolic acids together with minor ellagitannins. Depending on chromatographic and spectroscopic analyses, the isolated compounds were identified as gallic acid, methyl gallate, kaempferol, myricetin, ellagic acid 3-*O* - methyl ellagic acid, myricetin 3-*O*-( 4"-*O*- acetyl)- α- L- rhamnopyranoside (myricetin 4"- O- acetate, myricetrin, chlorogenic acid, quarcetin 4"-O- acetate, quarcetin 3-0- α - L rhamnopyranoside (quercet), kaempferol 3-O-β-D glucuronopyranoside and nilocitin (Mahmoud *et al.*, 2001). They also reported that leaves of *Eugenia jambolana* are rich in flavonols, glycosides and some of their acylated rhamnosides. Hewawasam *et al.* (2003) stated that flavonoids were known to be antioxidants, free radical scavengers and antiperoxidants and thereby hepatoprotective agents. Gupta and Sharma (1974) isolated sitosterol, betulinic acid, crategolic (maslinic) acid, hepatcosane, n- nonacosane, n-hentriacontane, n- octacosanol, n- triacontanol n- dotricontanol and sugars like glucose, fructose and acids like oxalic, citric and glycolic acids. Aminoacids glycine, alanine, tyrosine and leucine were also isolated from the leaves of *Eugenia jambolana.* Timbola *et al.* (2002) isolated quarcetin,

myricetin, myricitrin and a flavonols glycoside myricitrin and a flavonols glycoside myricetin 3-O- 4"- acetyl) - α - L rhamnopyranoside from its leaves. The leaves also contain some essential oils.

Pepato *et al.* (2001) observed no significant difference in biochemical and physiological parameters when decoction of *Eugenia jambolana* leaves was given to Streptozotocin- induced diabetic rats as a substitute for water. From this they concluded that the leaves have no antidiabetic activity.

Ravi *et al.* (2004) reported that oral administration of ethanolic extract of *Eugenia jambolana* seed kernel in Streptozotocin- induced diabetic rats, significantly decreased the levels of glycosylated haemoglobin, increased the body weight and haemoglobin, and restored the activities of superoxide dismutase, catalase, and glutathione peroxidase to the normal levels because of their antioxidant property. Silva *et al.* (2006) also reported about the antioxidant compounds in the leaves of *Eugenia jambolana.* The hepatoprotective effect of the plant *Eugenia jambolana* may be attributed to the presence of phytochemical constituents like phenolic compounds, flavonoids and tannins, mainly through their antioxidant activity.

Flavonoids, which occur both in the free state and as glycosides, are the largest group of naturally occurring phenols and have broad biological properties. The capability to interact with protein phosphorylation and the antioxidant, iron chelating and free radical scavenging activity may account for the wide pharmacological profile of flavonoids. The scavenging of free radicals by *Syzygium cumini* leaves extract may have played an important role in providing the protection against the radiation- induced damage to DNA (Jagetia *et al.*, 2002).

The aqueous leaf extract of *Eugenia jambolana* contained several polyphenolic compounds and flavonoids which were responsible for the antiviral activities against buffalo pox virus (Bhanuprakash *et al.*, 2007).

The *Syzygium cumini* leaves extract has antimicrobial activity due to the tannins and other phenolic constituents and is very rich in gallic acid and ellagic acid polyphenols derivatives (Oliveira *et al.*, 2007)

## 5.2 PHYSIOLOGICAL PARAMETERS

### 5.2.1 Body weight

In the present study, all the groups except the paracetamol treated group showed a gradual increase in weight gain. Paracetamol treated group showed a weight loss after the paracetamol administration, which may be due to the reduced feed intake and also the animals were under the stress of the hepatotoxic agent. Mathew (2005) also observed the weight loss in rats, which were treated with paracetamol at the dose of 3g/ Kg orally for three days and these results were comparable.

## 5.3 OBSERVATIONS

### 5.3.1 Antioxidant effects

The leaves of the plant contain polyphenols and flavonoids which were proven antioxidants.

#### *5.3.1.1 Superoxide dismutase*

Increase in serum activity of Superoxide dismutase (SOD) is a sensitive index in hepatocellular damage. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anions to form hydrogen peroxide, hence 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 present study, SOD levels were decreased in paracetamol treated groups which correlates with the findings of Gupta *et al.* (2004) and Murugesh *et al.* (2005). Treatment with ethanolic extract of *Eugenia jambolana* leaves showed an increase in SOD activity in a dose dependent manner. The enzymic antioxidant defense system is the nature's protection against lipid peroxidation. SOD, catalase and glutathione peroxidase are important scavengers of superoxide ion and hydrogen peroxide (Dash *et al.*, 2007).

When heavy dose of paracetamol was administered the animal was under stress and the level of SOD was decreased. The highest SOD value was noticed in the silymarin treated animals.

This showed that ethanolic extract of *Eugenia jambolana* can reduce the reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of hepatic antioxidant enzyme.

*5.3.1.2 Catalase*

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and highest activity is found in the red cells and liver. Catalase decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Dash *et al.*, 2007).

In the present investigation antioxidant enzymes like SOD and catalase levels were low with the paracetamol treated group. Similar results were also obtained by Iwalokun *et al.* (2006) with the administration of acetaminophen at the dose of 300mg/Kg intraperitoneallly for seven days. Treatment groups showed an increase in the catalase level at the doses 100 and 200mg/Kg. At the dose rate of 200mg/Kg the effects could be compared with the silymarin because no significant difference exist in between them ($P<0.05$). As reported earlier the phenolic compounds, flavonoids and the tannins present in the extract may be responsible for this property because of their antioxidant effect.

Administration of ethanolic extract of *Eugenia jambolana* increased the activities of SOD and catalase in paracetamol induced liver damage in rats to prevent the accumulation of excessive free radicals and protects the liver from paracetamol intoxication.

## 5.4 SERUM PARAMETERS

The study of different enzyme activities such as Alanine amino transferase (ALT), Aspartate amino transferase (AST), Serum alkaline phosphatase (SALP), total protein

and total bilirubin have been found to be of great value in the assessment of clinical and experimental liver damage. Kaushal *et al.* (1999) observed six fold increase in AST and eight fold increase in ALT levels in paracetamol treated rats.

### 5.4.1 Alanine aminotransferase (ALT)

Serum transaminase levels give a reliable indication of liver damage (Dixon *et al.*, 1975). Serum glutamate pyruvate transaminase, which is also known as alanine amino transferase (ALT) is a specific liver marker enzyme (Bose *et al.*, 2007) and when there is damage to the liver cells, the cell integrity will be lost and increased levels of these enzymes were released in to blood. A very high ALT level is seen in paracetamol poisoning cases.

In the present study, an increased level of ALT was observed in the paracetamol treated rats than the other groups, which signifies the ability of paracetamol to induce hepatotoxicity. The ethanolic extract at both the doses reduced the increased level of the enzyme and it was dose dependent. There was no significant difference in the enzyme levels noticed between the normal animals and silymarin treated animals. These results can be compared with the results obtained by Bose *et al.* (2004). They studied the antioxidant and hepatoprotective effect of *Eupatorium ayapana* in $CCl_4$ induced toxicity and found that at the dose of 200 mg/Kg it can give significant hepatoprotection.

### 5.4.2 Aspartate aminotransferase (AST)

This is also a liver specific enzyme. An elevated level of SGOT is always noted along with increased level of SGPT, which play a vital role in the conversion of amino acids to keto acids (Sallie *et al.*, 1999).

High levels of AST indicate liver damage, cardiac infarction and muscle injury. (Mc.Gregor *et al.*, 2003) In the present study it was demonstrated that *Eugenia jambolana* ethanolic extract at the doses 100 and 200 mg/Kg caused significant decrease of ALT and AST in a dose dependent manner. These results were similar as that of

Moresco *et al.* (2007) and they reported that at the dose of 0.9g/Kg aqueous extract of *Syzygium cumini* leaves for seven days, significantly decreased the SGOT and SGPT activities in the pretreated groups. They also opined that the scavenging of free radicals by the *Syzygium cumini* may be playing an important role in providing the protection against the hepatotoxicity induced by $CCl_4$.

### 5.4.3 Total protein

Protein synthesis is one of the functions of the liver. When the liver is damaged a reduction in protein synthesis will be noticed. A reduction in total serum protein and liver glycogen were observed in the paracetamol treated group which may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and glycogen. Consequently a decrease in liver weight may occur (Gupta *et al.*, 2006). Hypoproteinaemia is common in advanced chronic liver diseases. Here the paracetamol treated rats have the least total protein level. In the extract treated groups the level is almost similar to the other groups. Methanolic extract of *Pterocarpus marsupium* at the dose rate of 25mg/Kg/day for 14 days orally restored the levels of serum protein, bilirubin and the marker enzymes in hepatotoxicity induced by carbon tetrachloride (Mankani *et al.*, 2005). The ethanolic extract of *Eugenia jambolana* has not much significant effect on the total protein level.

### 5.4.4 Serum albumin

Serum albumin is a marker of synthetic function of the liver and is a valuable guide to asses the severity of chronic disease. It is the major component in the total protein. Albumin was significantly decreased in the paracetamol alone treated groups, while the decrease observed in the extract treated groups was lesser than the former group. This observation can be compared with the findings of Shenoy *et al.* (2002). They reported that paracetamol at the dose of 2g/Kg produced liver damage in rats as indicated by a significant reduction of total protein and albumin. Rats treated with the extract have increased levels of total protein and albumin in serum when compared with the paracetamol alone treated group, which indicated its hepatoprotective activity.

Stimulation of protein synthesis has been advanced as a contributory mechanism, which accelerates the regeneration and production of liver cells (Antony *et al.*, 2006).

### 5.4.5 Serum total bilirubin

Bilirubin is an yellow pigment produced by enzymatic degradation of haeme. Serum bilirubin levels are related to the function of hepatic cell. The clinical signs of icterus or jaundice develops when the yellow pigment bilirubin accumulate in plasma and other tissues. Bilirubin in serum can be assessed by the Vanden Bergh or diazo reaction, in which bilirubin is coupled with diazotized sulfanilic acid. Prolonged destruction of hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes an elevation in the serum bilirubin, ALP and LDH (Bose *et al.*, 2007). The rise in the levels of serum bilirubin confirms the hepatotoxicity which is induced by the paracetamol. Gupta *et al.* (2006) also reported that paracetamol at the dose of 200mg/Kg orally as a single dose increased the level of serum ALT, AST, ALP and bilirubin. The highest value was noticed in the paracetamol alone treated group. Results were in agreement with the observations of Roy *et al.* (2006). They reported an increase in bilirubin level when paracetamol was administered at the dose of 1g/Kg in three divided doses. All the other groups except the paracetamol treated group did not differ significantly ($P<0.05$). The bilirubin value is restored in animals treated with the extract at both the doses. So it can be inferred that it has hepatoprotective effect.

## 5.5 HAEMATOLOGICAL PARAMETERS

Haematological parameters like haemoglobin concentration, total leukocyte count (TLC) and differential leukocyte count (DLC) was studied before and after the treatment. In the case of hemoglobin concentration, total leukocyte count and differential leukocyte count, no change noticed before and after the treatment.

## 5.6 GROSS AND HISTOPATHOLOGICAL EXAMINATION OF LIVER

After ten days of treatment all the animals were sacrificed and liver was subjected to gross examination. Gross examination of the liver from all the groups except the paracetamol treated groups showed almost normal appearance and colour. Liver of the paracetamol alone treated rats showed diffuse coagulation necrosis (Fig.10). Hewawasam *et al.* (2003) observed that the liver of the paracetamol intoxicated mice appeared dark and congested. Bhanwra *et al.* (2000) reported that liver of most of the paracetamol treated rats were covered with white slough and multiple white patches indicating necrosis.

On histopathological examination the control group showed portal triad, rows of hepatocytes and normal arrangements of hepatocytes. The paracetamol treated group showed only a few areas of normal hepatocytes and extensive areas of coagulation necrosis. The liver showed confluent necrosis with vacoulation and ballooning degeneration in the surviving hepatocytes after treatment with paracetamol according to Hewawasam *et al.* (2003). Paracetamol at the dose of 2.5g/Kg orally produced marked congestion, central venous dilatation and the surrounding sinusoids were disrupted and packed with red blood cells after 48 hours of administration. Frank necrosis of liver parenchyma was also noticed by Dixon *et al.* (1971). It was reported that the earliest detectable change is chromatolysis (loss of basophilic granules) in the centrilobular cells. Mathew (2005) reported that the paracetamol at the dose rate of 3g/Kg for three days resulted in centrilobular coagulative necrosis of the liver. These results were comparable. The covalent binding of the oxidation products of the paracetamol, to the sulfhydryl groups of the protein resulting in the cell damage and thereby necrosis of the liver (Mathew, 2005).

In the 100mg/Kg extract treated group liver showed only diffuse necrotic areas involving only certain lobules. The hepatocytes in the surrounding areas were hypertrophic. A few mononuclear cells could be seen in the necrotic area and the Kupffer cell reactions were prominent. In the 200mg/Kg extract treated group necrosis involving

only a few hepatocytes in certain lobules was noticed. The hepatocytes seen around such lesions were hypertrophic and some of the hepatocytes have hyperchromatic nuclei. There are indications of regeneration of the tissues indicating the positive effects of the extract on the liver parenchyma. It has been seen that the pretreatment with the extract reduced the damage which was induced by the paracetamol proving its hepatoprotective effect. It may be due to the free radical scavenging effect of the phenolic components, flavonoids and tannins present in the leaf extract. Moresco *et al.* (2007) opined that the scavenging of free radicals by the *Syzygium cumini* may be playing an important role in providing the protection against the hepatotoxicity. *Azadirachta indica* aqueous leaf extract itself could act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes and also reduced the elevated levels of serum enzymes by its membrane stabilizing action according to Chattopadhyay (2003).

The results of the present study revealed that the ethanolic extract of leaves of *Eugenia jambolana* has hepatoprotective activity in paracetamol induced hepatotoxicity in rats.

# *Summary*

## 6. SUMMARY

The present study was undertaken to evaluate the hepatoprotective effect of ethanolic extract of *Eugenia jambolana* (Njaval) leaves on paracetamol induced hepatotoxicity in rats.

Fourty adult male wistar rats weighing 150-200 g, divided into five groups comprising eight animals in each group, were used for the study. Groups I and II animals were administered with 3 percent gum acacia suspension in distilled water at the dose rate of 5ml/Kg/ day for ten days. Groups III and IV animals received ethanolic extract of *Eugenia jambolana* leaves at the dose of 100 and 200 mg/Kg/ day in 3% gum acacia for ten days. Group V animals received standard hepatoprotective drug silymarin at the dose of 100 mg/ Kg/day in 3% gum acacia for ten days. All the groups except the group I, received paracetamol orally on the eighth day at the dose rate of 2g/Kg.

Blood was collected from all the groups prior to the experiment and fourty eight hours after the paracetamol administration for haematological examination and serum was separated for biochemical studies. All the animals were sacrificed on the $10^{th}$ day and liver was taken estimation of superoxide dismutase and catalase and for histopathological examination. Body weight was recorded on day 0 and $10^{th}$ day. Serum was used for the estimation of ALT, AST, total protein, albumin and bilirubin.

Phytochemical analysis of the plant extract revealed the presence of phenolic compounds, flavanoids, tannins, glycosides, diterpenes and triterpenes.

Paracetamol administered group showed a decrease in body weight while all others showed an increase in body weight except the 100mg/Kg *Eugenia jambolana* extract treated animals which showed no change.

A decrease in the levels of SOD and catalase were noticed in the paracetamol alone treated rats. Administration of ethanolic extract of *Eugenia jambolana* at both the doses brought about a significant increase in the level of these antioxidant enzymes

which proved its ability to reduce the oxidative stress and liver damage. But it was dose-dependent and the higher dose (200mg/Kg) is found more effective.

Liver marker enzymes like ALT, AST levels can be used for the assessment of the intensity of hepatotoxicity. Both these enzymes were highest in paracetamol treated groups. The elevated levels of these enzymes were decreased by the herbal extract in a dose dependent manner and thereby proved their hepatoprotective activity.

A reduction in the total protein was observed in the paracetamol treated group. The herbal extract at both the doses maintained the total protein level like the normal animals. The level decreased in the paracetamol alone treated group was associated with the decrease in the number of hepatocytes, thereby decreased hepatic capacity to synthesize protein. Serum albumin level was also lowest in the paracetamol treated group because of the decrease in the total protein level. Serum albumin level is a marker of synthetic function of the liver and is a valuable guide to asses the severity of chronic diseases. The extract at both the doses increased the serum albumin level in a dose dependent manner.

Serum bilirubin level was also highest in the paracetamol treated group because of the liver damage caused by the paracetamol. The extract at the dose of 100 and 200 mg/Kg level decreased the elevated level of bilirubin.

The study of haematological parameters like haemoglobin concentration, total leukocyte count and differential leukocyte count revealed that they were not much affected with the extract treatment.

Gross examination of the liver showed normal appearance in all the four groups except the paracetamol treated group. In the paracetamol treated animals the liver showed areas of coagulative necrosis and congestion. On histological examination the paracetamol treated group showed extensive areas of coagulation necrosis. The *Eugenia jambolana* treated animals showed diffuse necrotic areas in certain lobules and hypertrophy of hepatocytes and regeneration. Silymarin treated animals have almost normal liver architecture like the control group, except some mild foci of necrosis.

In the present study, the ethanolic extract of *Eugenia jambolana* leaves showed a significant hepatoprotective action in a dose dependent manner by increasing antioxidant enzymes like SOD and catalase. It decreased the toxic effects of paracetamol in the liver by the scavenging of free radicals and thereby reduced the oxidative stress and found that the higher dose is found more effective. Phytochemical potent antioxidant constituents *viz.* phenolic compounds, flavanoids and tannins present in the leaves may be responsible for the hepatoprotective effect.

From the present study it can be concluded that the ethanolic extract of *Eugenia jambolana* has significant hepatoprotective activity in a dose dependent manner in paracetamol induced hepatotoxicity.

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# EVALUATION OF HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *Eugenia jambolana* (NJAVAL) LEAVES ON PARACETAMOL INDUCED TOXICITY IN RATS

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## ABSTRACT

The present study is undertaken to evaluate the hepatoprotective effect of *Eugenia jambolana* (Njaval) leaves on paracetamol induced toxicity in rats.

Fourty adult male albino wistar rats weighing 150-200 g were randomly divided into five groups of eight animals each. Group I received 5 ml/Kg/day of 3 % gum acacia (vehicle) orally daily for ten days. Groups III and IV received ethanolic extract of *Eugenia jambolana* leaves at the dose rate of 100 and 200 mg/Kg/day respectively in 3% gum acacia for ten days. Group V received silymarin 100 mg/Kg/day in 3% gum acacia for ten days. Hepatotoxicity was induced in groups II, III, IV and V by giving a single dose of paracetamol at the dose of 2g/Kg on the eighth day of the experiment. Blood was collected before and after the experiment for various biochemical and haematological parameters. Body weight of all the animals was recorded on the first day and last day. On the tenth day all the animals were sacrificed and liver was collected for biochemical and histopathological studies.

Paracetamol treated rats showed a decrease in their body weight. Biochemical parameters like superoxide dismutase and catalase were also decreased in the paracetamol treated groups when compared with the others. Liver marker enzymes like ALT, AST were increased in the paracetamol treated groups, but the treatment with extract at both the doses reduced the elevated level of these enzymes. The total protein level and albumin level were decreased in the paracetamol treated groups and a gradual increase was noticed in the extract treated groups. The total bilirubin level was highest in the paracetamol alone treated group and less in the extract treated groups. Haematological parameters showed not much significant change with the treatment.

Phytochemical analysis of the plant extract revealed the presence of phenolic compounds, flavonoids, tannins, glycosides, diterpenes and triterpenes. All the animals showed normal liver except the paracetamol treated group. In the paracetamol treated group areas of necrosis was noticed. On histological examination, the paracetamol treated

group showed areas of coagulation necrosis and the treatment groups showed regeneration of the hepatocytes.

The study revealed that the ethanolic extract of leaves of *E. jambolana* at the doses 100 and 200 mg/Kg/day have significant hepatoprotective activity in paracetamol induced toxicity in rats.