NEPHROPROTECTIVE EFFECT OF *Aerva lanata* (Cherula) AND *Boerrhavia diffusa* (Thazhuthama) 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 **"NEPHROPROTECTIVE EFFECT OF *Aerva lanata* (Cherula) AND *Boerrhavia diffusa* (Thazhuthama) IN RATS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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23.09.03

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CERTIFICATE

Certified that the thesis entitled **"NEPHROPROTECTIVE EFFECT OF *Aerva lanata* (Cherula) AND *Boerrhavia diffusa* (Thazhuthama) IN RATS"** is a record of research work done independently by **Dr. Fakrudeen Ali Ahamed. N.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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Fakrudeen Ali Ahamed. N.

DEDICATED TO THE ALMIGHTY

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Introduction

1. INTRODUCTION

Nephrotoxicity is a major toxic effect of antibiotics in both animals as well as human beings. Broad-spectrum antibiotics like aminoglycosides are commonly used for the treatment of various infectious diseases.

Nephrotoxicity is the major side effect of aminoglycosides, especially gentamicin, accounting for 10 to 15 per cent of all cases of acute renal failure (Homes and Weinberg, 1986). The specificity of gentamicin renal toxicity is apparently related to its preferential accumulation in the renal convoluted tubules and its effect on biological membranes. Based on the fact that kidneys are the major routes of drug excretion, the occurrence of nephrotoxicity is relatively frequent.

Many different chemical agents are used to prevent nephrotoxicity in both animal models and human subjects. The use of hydroxyl radical scavengers might mitigate gentamicin induced kidney function in rats (Walker and Shah, 1988).

The aminoacids polyasparagine and polyaspartic acid have been demonstrated to possess nephroprotective effect against gentamicin induced nephrotoxicity (Gilbert et al., 1989). The growing awareness of the secondary effects of these nephroprotective agents, made people to explore the time tested remedies from traditional alternative medicine.

"Ayurveda", an indigenous system of medicine, offers wide scope for the treatment of nephrotoxicity. It is estimated that 80 per cent of people in developing countries are almost completely dependent on traditional healers for their health care and that plants are the major source of drugs for these traditional medical practitioners. It is argued that since 80 per cent of the world's population live in the developing countries, about 64 per cent of the world's population

therefore depends almost entirely on plants for medication (Fransworth and Soejarto, 1985).

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last five year period (Dahanukar *et al.*, 2000).

The main reason for herbal drug usage is the belief that these drugs have lesser side effects. India, being a tropical country, is blessed with vast natural resources and the ancient knowledge for the effects claimed for various remedies.

In order to make these traditional remedies acceptable to the modern physicians, there is a need to evaluate them to identify the active principles and to understand the mechanism of action of the drug.

In the present study, an attempt has been made to validate the folklore use of *Aerva lanata* (Cherula) and *Boerrhavia diffusa* (Thazhuthama) as nephroprotective agents against experimentally induced nephrotoxicity in rats.

The objectives of the study are:

1. To study the nephroprotective effect of *Aerva lanata* (Cherula) and *Boerrhavia diffusa* (Thazhuthama) against the gentamicin induced nephrotoxicity in rats.

2. Assessment of effect of *Aerva lanata* and *Boerrhavia diffusa* in blood urea nitrogen level.

The information gathered by the study would help in suggesting these plant preparations as nephroprotective agents.

Review of Literature

2. REVIEW OF LITERATURE

2.1 NEPHROTOXICITY

Black *et al.* (1963) reported that dogs were given gentamicin at the dose rate of 40 mg/kg/day intramuscularly for 15 days became moribund and were euthanized, the necropsy revealing extensive renal proximal tubular necrosis.

Cats when administered 50 mg/kg/day of gentamicin for 65 days, had markedly elevated serum urea nitrogen concentrations. In six cats given 35 mg/kg/day of gentamicin, intramuscularly, the serum urea nitrogen concentrations were 50 mg/dl or greater after 6-13 days. Renal tubular necrosis was present on histopathological examination (Welles *et al.*, 1973).

Luft *et al.* (1977) investigated that in the rat, the concomitant administration of gentamicin did not interfere with recovery from mercuric chloride induced renal failure. Rats recovering from mercuric chloride induced acute renal failure were resistant to a depression in glomerular filtration when given gentamicin. The prior administration of gentamicin enhanced the nephrotoxicity of mercuric chloride.

Fry *et al.* (1977) stated that gentamicin at normal clinical dose produced greater, more rapid elevations of the blood urea nitrogen, although all elevations were reversible.

Sairio *et al.* (1978) reported that the critical kidney damaging concentration of free aminoglycosides in the whole rat kidney was estimated to be 160-190 µg/g body weight.

Dantas *et al.* (1997) investigated that dogs received gentamicin at the dose rate of 10 mg /kg body weight intramuscularly, 3 times a day for 14 days showed loss of appetite, apathy, polyuria, polydipsia, diarrhoea, vomition and oliguria. Laboratory findings included enzymuria, crystalluria, azotaemia, and isosthenuria. Gross lesions were restricted to the kidneys, which were pallid, swollen, and soft. Histologically, tubular necrosis, restricted to the proximal convoluted tubules was observed.

Leukocyte count, absolute neutrophil count and serum tri iodothyronine decreased and serum creatinine and blood urea nitrogen concentrations increased in guinea pigs following intramuscular administration of gentamicin at the dose rate of 4 mg/kg body weight for 7 days. No significant effect on packed cell volume, total erythrocyte count, haemoglobin concentration, serum thyroxine and total serum protein, were observed. Histopathology revealed mild nephrotoxic lesions in kidneys after gentamicin administration (Gupta and Verma, 1998).

Administration of graded doses of gentamicin to adult wistar strain rats at doses of 20, 40 and 80 mg/kg/day for 7 days induced nephrotoxicity exhibited by elevated plasma creatinine and urea concentration (Bajpai *et al.*, 1999).

Long term exposure of gentamicin in the renal proximal tubule-like cell line, LLc-pk1 induces apoptosis of the renal tubular epithelial cells and this process might contribute to some of the aminoglycosides nephrotoxicity (Choi *et al.*, 2000).

Vijaykumar *et al.* (2000) confirmed that gentamicin at a dose rate of 80 mg/kg/day subcutaneously for 12 days in rats produced significant nephrotoxicity as evidenced by renal tubular necrosis, increase in blood urea, serum creatinine and decreased creatinine clearance.

Ali *et al.* (2001) concluded that a sex related difference in the susceptibility of Sprague-Dawley rats to gentamicin nephrotoxicity exists and that treatment with sex hormones (2 mg/kg/day for 15 days) did not significantly affect the toxicity.

Bhalerao *et al.* (2001) found that nephrotoxicity was induced by gentamicin at the dose rate of 80 mg/kg/day subcutaneously for three days in adult male albino rats.

2.2 NEPHROPROTECTIVE AGENTS

Meister (1981) reported that glutathione peroxidase (GSH) was responsible for protection of cells against oxidative stresses.

Trease and Evans (1985) stated that flavanoids have been shown to possess diuretic activity in rats.

Urinary alkalinization seemed to be an effective method for the prevention of gentamicin nephrotoxicity in rats (Lee *et al.*, 1988).

Walker and Shah (1988) reported that the use of hydroxyl radical scavengers might mitigate gentamicin induced kidney functions in rats.

Gilbert *et al.* (1989) confirmed that aminoacids polyasparagine and polyaspartic acid have been demonstrated to possess nephroprotective effect against gentamicin induced nephrotoxicity in rats.

Thompson *et al.* (1990) concluded that gentamicin nephrotoxicity can be reduced in rats by the loading of potassium chloride (3.5% potassium diet).

Sandhya *et al.* (1995) concluded that administration of DL alpha-lipoic acid (25 mg/kg/day) to rats prevents lipid peroxidation which may atleast partly, play an important role in the injury cascade of gentamicin induced nephrotoxicity.

Naim *et al.* (1999) reported that due to their antioxidant activity vitamin-E (250 mg/kg) and probucol (60 mg/kg.) given intramuscularly have potential protective effects in rats against gentamicin nephrotoxicity.

Mune at al. (1999) concluded that progression of glomerular sclerosis in the rat remnant kidney model of progressive kidney disease could be significantly modulated with five per cent dietary fish oil treatment.

Rao *et al.* (1999) reported that cystone® (1000 mg/kg body weight) protects against cisplatin induced nephrotoxicity in mice without interfering with its antitumor activity.

Quercetin, a plant bioflavanoid was found to possess significant cytoprotective effect on cisplatin induced renal tubular damage invivo in rats at the dose rate of 20 mg/kg intraperitonially. Cisplatin, which produced renal toxicity by increasing lipid peroxidation was found to markedly increase the levels of lipid peroxides, thiobarbituric acid reactive substances (TBARS) and decrease the level of antioxidant enzymes like superoxide dismutases (SOD), catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase. Quercetin pretreatment was found to reverse all these changes (Devipriya and Shymaladevi, 1999).

Vijaykumar *et al.* (2000) evaluated that co-administration of probucol (10 mg/kg body weight) orally with gentamicin prevents both functional and histological renal changes induced by gentamicin in rats. He also noticed blood

urea nitrogen and serum creatinine was increased by 963 and 462 per cent respectively with gentamicin compared to saline treated animals.

Malini *et al.* (2000) reported that treatment with lupeol and betulin at the dose rate of 35 mg/kg body weight/day orally for 15 days in rats restored the levels of various anti-oxidant enzymes and the glutathione related enzymes in both kidney and bladder.

Vitamin C at the dose rate of 50, 100, 200 mg/kg body weight showed nephroprotection in a dose dependent manner on cisplatin induced oxidative damage on adult Wistar rat kidneys (Lusania *et al.*, 2000).

Ali and Mousa (2001) found that Dimethyl sulfoxide (DMSO) dose dependently lowered the elevated plasma urea and creatinine concentrations and the rise in cortical thiobarbuturic acid reactive substances (TBARS) and suggested that Dimethyl sulfoxide (25%) has potential protective effect against gentamicin nephrotoxicity in rats.

Buyukafsar *et al.* (2001) stated that, trapidil at a higher dose (20 mg/kg body weight) protected kidney against gentamicin nephrotoxicity in rats.

Paquette *et al.* (2002) demonstrated chronic gentamicin induced renal toxicity varies temporarily according to the time of administration and that a mixed protein diet containing a lower fat level protected kidney against gentamicin induced nephrotoxicity in rats.

Sener *et al.* (2002) observed that simultaneous melatonin administration in rats at the dose rate of 10 mg/kg body weight protected kidney tissue against the oxidative damage and the nephrotoxic effect caused by gentamicin treatment.

Antus *et al.* (2003) stated that estrogens prevented the development of glomeruloscelerosis in the rat remnant kidney model.

2.3 NEPHROPROTECTIVE PLANTS

Chopra *et al.* (1956) stated that the fruit pulp of *Lagenaria siceraria* possess cooling, diuretic and antibilious properties.

Prasad *et al.* (1993) proved the efficacy of Musa (Paradisiaca Linn. cultivar) stem juice at the dose rate of 3 ml/rat/day orally in reducing the incidence of urolithiasis in albino rats to a considerable extent, thus justifying the claim made in the indigenous system of medicine.

Prasad *et al.* (1994) proved the efficacy of ethanolic extract of *Ammannia baccifera* Linn. as anti- urolithic as claimed in the indigenous system of medicine.

Anand *et al.* (1994) found that ethanolic extract of the fruits of *Tribulus terrestris* showed significant dose dependent protection against uroliths induced by glass bead implantation in albino rats. On subsequent fractionation of this extract maximum activity was localized in 10 per cent aqueous methanolic fraction.

Srividya and Periwal (1995) reported that *Phyllanthus amarus* was a potential diuretic, hypotensive and hypoglycemic drug for humans.

Herrera *et al.* (1998) stated that the water soluble fraction of the methanolic extract of the defatted dried ground whole plant of *Vernonic cinera* (Sahadeva) showed significant diuretic activity in rats comparable to furosemide (Lasix®) a known diuretic.

Banadequl Buzoor, a celebrated Unani formulation containing 13 medicinal plants, reported to be nephroprotective. Banadequl Buzoor decreased the serum urea and creatinine level in rats significantly, that were increased by gentamicin administration at the dose rate of 40mg/kg body weight twice a day for seven days (Shamim *et al.*, 1999).

Naidu *et al.* (2000) found blood urea and serum creatinine were increased by 896 per cent and 461 per cent respectively with gentamicin, compared with the control. Changes in blood urea, serum creatinine and creatinine clearance induced by gentamicin were significantly prevented by the extract of *Ginkgo biloba* at the dose rate of 300 mg/kg body weight orally. Thus they concluded, *Ginkgo biloba* extract protected rats from gentamicin induced nephrotoxicity.

Tahri *et al.* (2000) concluded that an acute hypotensive action of *Urtica dioica* indicated a direct effect on the cardiovascular system. Moreover a diuretic and natriuretic effect were also observed in rats at a high dose of 24 mg/kg body weight suggesting an action on the renal function.

Chaverry *et al.* (2000) stated that reactive oxygen species were involved in gentamicin nephrotoxicity. The protective effect of garlic (2% garlic diet) in rats was associated with the prevention of decrease of glutathione peroxidase (Gp_x) activities and with the rise of lipoperoxidation in renal cortex.

Curcumin protected against adriamycin induced renal injury in rats by suppressing oxidative stress, increasing kidney glutathione content and glutathione peroxidase activity, curcumin abolished adriamycin stimulated kidney microsomal and mitochondrial lipid peroxidation, thus curcumin restored renal function (Narayanan *et al.*, 2000).

Perez *et al.* (2000) evaluated that aqueous extracts of the heartwood of *Eysenhardtia polystachya* possess antilithiatic and diuretic activity at the dose rate of 0.5, 1.0 and 2.0 mg/kg body weight in rats.

Liu *et al.* (2000) described that pretreatment of mice for two days with *panar notoginseng* saponins at 100 or 200 mg/kg body weight suppressed cisplatin induced high blood urea nitrogen level to 83 and 31 per cent serum creatinine level to 86 and 42 per cent respectively.

Kumar *et al.* (2001) investigated that 50 per cent ethanolic extract of the whole plant of *Solanum nigrum* exhibited significant hydroxyl radical scavenging potential, thus suggesting its probable mechanism of cytoprotection against gentamicin induced kidney cell (vero cells) damage.

Meher *et al.* (2001) observed that indigenous drugs Gokshura *(Tribulus terrestris)* at the dose rate of 130 mg/kg body weight and varun (*Crataeva nurvala* Buch Ham*)* at the dose rate of 1450 mg/kg body weight orally have nephroprotective action against gentamicin induced nephrotoxicity in albino rats.

Bhalerao *et al.* (2001) stated that kurchi was effective in rats in preventing nephrotoxicity induced by drugs such as gentamicin.

Samiulla and Harish (2001) revealed that co-administration of both the formulations NR-AG-1 (containing *Crataeva nurvala, Tribulus terrestris, Dolichos biflorus, Shilajeet)* and NR-AG-II *(Crataeva nurvala, Boerrhavia diffusa, Saccharum officinarum, Butea frondosa*) at the dose rate of 150 mg/kg/day orally along with gentamicin at the dose rate of 80 mg/kg/day subcutaneously prevented both functional and histological renal changes induced by gentamicin in rats.

Arabic gum at the dose rate of 7.5 g/100 ml orally protected the rats from gentamicin induced nephrotoxicity, possibly atleast in part through inhibition of the production of oxygen free radicals that cause lipid peroxidation (Majed *et al.*, 2002).

Ali (2002) reported that crude water extract of *Rhazya stricta* leaves at the dose rate of 0.5 and 1 g/kg body weight orally contain compounds that could potentially ameliorate gentamicin nephrotoxicity in rats.

Ali *et al.* (2003) reported that *Tribulus terrestris* had long been used empirically to propel urinary stones. The diuretic and contractile effects of *Tribulus terrestris* (5 g/kg body weight orally) in rats indicated that it has the potential of propelling urinary stones.

2.4 *Aerva lanata*

Udupihille and Jiffry (1986) stated that infusions of the herb, *Aerva lanata* was popular in our country as a home remedy for the treatment of urinary infections, but the properties of the aqueous extract of the herb had never been investigated until recently. They found that an extract of the whole herb produced a significant diuresis when compared to water and to isotonic saline and also that an infusion of only the fresh flowers and leaves of the herb produced a much more intense diuresis than that produced by the whole plant with a maximum urine flow at 15 mts.

Aerva lanata (family – Amaranthaceae) is commonly known as 'Chaya', called as 'Cherula' in Malayalam, 'Sirupoolai' in Tamil. It grows as a weed throughout the plains of India and the whole plant is used as diuretic (Chatterjee and Chandraprakash, 1992).

Goonaratna *et al.* (1993) stated that *Aerva lanata* extract did not produced any gastro intestinal adverse effects in healthy volunteers.

Majmudar *et al.* (1999) reported that the alcoholic extract of *Aerva lanata* administered at the dose rate of 1600 mg/kg body weight orally to albino rats showed significant diuretic activity. Sodium output in urine was markedly increased, but there was little effect on potassium output.

Vetrichelvan *et al.* (2000) concluded that the alcoholic extract of *Aerva lanata* administered orally to rats at the dose rate of 800 mg/kg body weight increased the urine volume moderately, acted as a potent kaliuretic in dose dependent manner, justified the usefulness of this plant in the treatment of inflammation and renal dropsies.

Increased urinary excretion of calcium oxalate, uric acid, phosphorus and protein in hyperoxaluric rats was brought down significantly by the oral administration of *Aerva lanata* extract at the dose rate of 3.0 mg/kg body weight (Selvam *et al.*, 2001).

Vetrichelvan and Jegadeesan (2002) reported that the ability of aqueous extract of *Aerva lanata* (500 mg/kg body weight) to protect the body weight loss seems to be due to its anti-diabetic activity and also the blood urea levels of extract treated rats indicated the partial inhibition of alloxan renal toxicity.

Phytochemical studies on *Aerva lanata* revealed that, it contains Aerva flavanoid glycosides, alpha and beta amyrin, betulin, campesterol, chrysin, narcissin, beta sitosterol, aervitrin, aervolanine, aervoside, canthin-6-one, canthin-10-hydroxy-6-one, canthin-10 methoxy-6-one, carboline-1-propionic acid, feruloyl-tyramine and homo feruloyl vanillyl amine (Pervykh *et al.*, 1993).

2.5 *Boerrhavia diffusa*

The plant *Boerhaavia diffusa* known as 'Punarnava' in Sanskrit, 'Spreading hogweed' in English and 'Thazhuthama' in Malayalam, is a perennial vigorously growing weedy vine indigenous to India and Brazil. The earliest mention of this plant was seen in 'Charaka Samhita' (Charaka, 1949).

The leaves of the plant were found to be useful in inflammations, heart diseases, liver diseases, dropsy and poisoning of various origin. The roots were said to have diuretic, anti-inflammatory and anti-pyretic properties (Priyavrat, 1956).

Chopra *et al.* (1956) described the use of *Boerhaavia diffusa* in asthma, edema, anemia, jaundice, anasarca and as an antidote to snake poisoning. The roots of the plant was reported to have diuretic, laxative and expectorant properties,

Chopra *et al.* (1958) reported that the root of the plant was employed for many curative purposes including liver, gall bladder, renal, urinary disorders and also the alkaloidal extract of the plant could produce a distinct and persistent diuresis.

The active principle of *Boerhaavia diffusa* was a compound of alkaloidal nature called 'Punarnavine'. Large quantities of potassium nitrate and other salts contained in the plant might contribute to its diuretic effect. Intravenous injection of the alkaloid in cats produced distinct and persistent rise in blood pressure and diuresis (Chopra *et al.*, 1958).

Abraham (1975) reported that the plant *Boerhavia diffusa* as a whole was effective in jaundice, edema, blood pressure and acting as a diuretic in mild doses, it cured asthma and in high doses, it acted as an emetic.

Nadkarni (1976) opined that 1-4 drachms of the liquid extract from the plant *Boerhaavia diffusa* produced diuresis in cases of edema and ascites especially due to early liver, peritoneal and kidney conditions. The diuresis was said to be mainly due to the action of the alkaloid punarnavine.

Boerhaavia diffusa was included as one of the extensively investigated medicinal plant in India (Vohora, 1989). He recommended detailed investigations on its anti-inflammatory and diuretic properties.

Singh *et al.* (1991) in an experimental evaluation of possible teratogenic potential of *Boerhaavia diffusa* in albino rats, observed that the ethanolic extract of *Boerrhavia diffusa* at the dose rate of 250 mg/kg body weight orally was devoid of any teratogenic effect. The litter size and survival rate of fetuses was also the same as that of the controls.

The alcoholic extract of *Boerhaavia diffusa* at the dose rate of 500 mg/kg body weight possessed significant protective activity against carbon tetrachloride induced hepatic injury in rats as revealed by a decrease in the levels of AST, ALT, serum bilirubin and bromo sulphalein clearance time (Chandan *et al.*, 1991) the extract did not show any signs of toxicity upto an oral dose of 2 g/kg body weight in mice.

Srivastava *et al.* (1998) reviewed the chemistry, pharmacology and botany of *Boerhaavia diffusa* extracts and their isolates. They described various chemical constituents of *Boerrhavia diffusa* (punarnavine) and reported that some of them possessed hepatoprotective, adaptogenic, anti-fibrinolytic diuretic and anti-viral properties.

Geetha and Sangeetha (2000) conducted a controlled experimental study to assess the effect of ***Boerhaavia diffusa*** extract at the dose rate of 2.4 g/kg body

weight orally to ward off post surgical infection and mortality in albino rats. The results showed that the drug caused (1) normal maintenance of level of water intake and urine output after surgery, (2) maintenance of total and differential.WBC count after surgery and infection and (3) prevention of accumulation of peritoneal fluid and onset of gangrene.

The aqueous leaf extract of *Boerhaavia diffusa* was found to possess significant hypoglycemic activity against alloxan induced diabetes in rats at the dose rate of 100, 200 and 400 mg/kg body weight orally with peak activity at six hour, post administration (Chude *et al.*, 2001).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Study was conducted in 48 adult albino rats of both sexes weighing 150-250 g. Rats were maintained on identical feeding and managemental practices in the laboratory for one week before the commencement of study.

3.2 DRUGS

3.2.1 Experimental Drugs

3.2.1.1 Aerva lanata

The whole plant of *Aerva lanata* (Cherula) was procured locally and identified (Fig.1). The plants were cleaned, dried under shade, and pulverized to a coarse powder in an electrical pulverizer. The powder was extracted in Soxhlet extraction apparatus using ethanol. The extract thus obtained was kept open to facilitate complete evaporation of solvent and then kept under refrigeration. When 100 g of dried powder were extracted, it yielded 8.4 g of extract.

3.2.1.2 Boerrhavia diffusa

The whole plant of *Boerrhavia diffusa* (Thazhuthama) was procured locally and identified (Fig.2). The plants were cleaned, chopped into pieces, dried in shade and pulverized to a coarse powder using electrical pulverizer. It was then extracted using ethanol in a Soxhlet extraction apparatus. The solvent was removed completely by evaporation to a thick semi solid extract. 100g of *Boerrhavia diffusa* powder yielded 18.9 g of extract. The extract was kept under refrigeration till use.

3.2.2 Normal Saline

Saline was prepared in the laboratory by dissolving 0.9 g of sodium chloride in 100 ml of distilled water.

3.2.3 Gentamicin Sulphate

Gentamicin sulphate was purchased locally and administered subcutaneously at the dose rate of 80 mg/kg body weight to induce nephrotoxicity.

3.3 PREPARATION OF PLANT EXTRACTS FOR ORAL ADMINISTRATION

For 250 mg/kg dose rates, 500 mg of extract was suspended in 10 ml of five per cent gum acacia so that the suspension contained 50 mg of extract/ml.

For 500 mg/kg dose rates, 1000 mg of extract was dissolved in 10 ml of five per cent gum acacia, so that one ml contain 100 mg of extract.

3.4 EXPERIMENTAL DESIGN

a. The rats were divided into six groups and each group consisting of eight rats of either sex.

b. Control group (G1) was given normal saline only.

c. All the experimental animals except control group (G1) were given gentamicin sulphate at the dose rate of 80 mg/kg/day subcutaneously (s/c) for 12 days continuously and produced nephrotoxicity.

After 12^{th} day, the experimental groups (G3, G4, G5, G6) were given two selected doses (G3 and G5 – 250 mg/kg) and (G4 and G6 – 500 mg/kg) of dried alcoholic extract of the two plants orally using eustachian catheter for a period of another 21 days respectively, whereas gentamicin sulphate control (G2) was sacrificed on 12^{th} day to assess the nephrotoxicity.

Table 1. Treatment groups and their dose regimen

Plants	Amount of extract (mg/kg)	
Control	Normal saline (G1)	
Gentamicin sulphate Control	80 mg/kg/day s/c for 12 days (G2)	
Aerva lanata	250 (G3)	500 (G4)
Boerrhavia diffusa	250 (G5)	500 (G6)

G = Group

3.5 OBSERVATIONS

3.5.1 Feed Intake

The feed intake of rats of both the treated and control groups were recorded daily and the data was analysed.

3.5.2 Body Weight

The body weight of rats of both the treated and control groups were taken and recorded before the commencement of the experiment, at weekly intervals, during the experiment and at the end of the experiment and data was analysed.

3.5.3 *Biochemical Parameters*

3.5.3.1 Blood urea nitrogen level was estimated at 0 and 12^{th} day for gentamicin sulphate control group (G2) and at 0, 12^{th}, 19^{th}, 26^{th} and 33^{rd} day of the experiment in all other groups (G1, G3, G4, G5, G6).

3.5.3.2 Serum was used for the estimation of blood urea nitrogen by the non-enzymatic method (Ranjna, 1995).

Set up the tubes and proceeded as follows.

Table 2. Procedure for estimation of blood urea nitrogen:

	Test (ml)	Standard (ml)	Blank (ml)
Serum (diluted 1:100)	1	--	--
Standard	--	1	--
Distilled water	1	1	2
Colour reagent	2	2	2
Acid reagent	2	2	2

Mixed thoroughly and placed the tubes in boiling water bath for 20 minutes. Cooled and read the pink coloured solution at 520 nm using Spectrophotometer.

Urea concentration in test samples were calculated from the optical density (OD) reading as follows:

$$\text{Blood urea (mg\%)} = \frac{\text{OD of test}}{\text{OD of standard}} \times \frac{\text{Concentration of standard}}{\text{Effective volume}} \times 100$$

$$= \frac{\text{OD of test}}{\text{OD of standard}} \times \frac{0.01 \times 100}{0.01}$$

$$= \frac{\text{OD of test}}{\text{OD of standard}} \times 100$$

3.5.4 Microscopical Examination of Urine

Urine samples of individual groups were centrifuged at low speed and the deposits were examined microscopically for sediments, crystals, casts and cells in all groups (G1, G2, G3, G4, G5 and G6) at 12th and 33rd day.

3.5.5 Gross and Histopathological Examination of Kidney

The gross and histopathological lesions in kidney was studied by collecting kidneys at 12th day in gentamicin sulphate control group (G2) and 50 per cent from saline control group (G1) and at 33rd day in all other groups (G3, G4, G5, G6) and remaining 50 per cent from saline control group (G1).

3.5.5.1 Gross Lesions

The gross lesions in kidneys of treated groups were compared with the control group.

3.5.5.2 Histopathological Study

The histopathological examination of kidneys was done to assess the nephrotoxicity. Three mm thick pieces of kidney tissue were selected randomly from both the control and experimental groups of rats and fixed in 10 per cent formaline and processed through routine paraffin embedding process, stained with haematoxylin and eosin and studied the histopathology (Sheehan and Hrapchak, 1980).

3.5.6 Haematological Study

Blood samples were collected from the retro-orbital plexus of both the treated and control groups of rats at 12th day and 33rd day of the experiment for

the estimation of packed cell volume, haemoglobin concentration, total erythrocyte count, total leukocyte count and differential leukocyte count.

3.5.6.1 Packed Cell Volume

Packed cell volume was estimated by Wintrobe method (Benjamin, 1978).

3.5.6.2 Haemoglobin Concentration (Hb)

Haemoglobin concentration was estimated by acid Haematin method (Benjamin, 1978).

3.5.6.3 Total Erythrocyte Count (TEC)

Total red blood cells were counted by using haemocytometer (Benjamin, 1978).

3.5.6.4 Total Leukocyte Count (TLC)

Total leukocytes were counted by standard dilution technique using Thomas fluid and haemocytometer (Benjamin, 1978).

3.5.6.5 Differential Leukocyte Count (DLC)

Blood smears were prepared from freshly drawn blood (without anticoagulant) by using slide method. After staining with Wright's stain, differential leukocyte count was done by counting and classifying 200 leukocytes under oil immersion (Benjamin, 1978).

All the data were analysed statistically using the method suggested by Snedecor and Cochran (1980).

Results

4. RESULTS

The present study was undertaken to evaluate the nephroprotective effect of *Aerva lanata* and *Boerrhavia diffusa* at two different dose levels against gentamicin induced nephrotoxicity in rats. The results obtained were tabulated and presented in Tables 3 to 21.The datas were also analysed statistically and are given in Tables 22 to 26, Fig. 3 to 5 and Fig.13 to 20.

Both *Aerva lanata* and *Boerrhavia diffusa* have showed significant nephroprotective action at the dose rates of 250 and 500 mg/kg body weight against gentamicin induced nephrotoxicity in rats.

4.1 SALINE CONTROL (G1)

In this group, no other drug except saline was administered orally at the starting (0 day) to the end of the experiment (33rd day). The values on feed intake, body weight, blood urea nitrogen and haemogram were tabulated and presented in Tables from 3 to 6. Microscopical examination of urine, gross and histopathological examination of kidney were also studied.

4.1.1 Feed Intake

Feed intake was recorded and presented in Table 3. The minimum and maximum feed intake was 104 and 130 gm/day respectively. There was no great variation in the feed intake and showed no significant difference within the group at different period of time interval (Fig. 3).

4.1.2 Body Weight

The results on body weight was analysed statistically and presented in Table 22 and in Fig.4. The mean body weight at 0,12,19,26 and 33rd day were 198.75±6.93, 201.25±6.67, 200.63±6.64, 202.5±5.98 and 202.5±5.98 respectively. There was no variation in the mean body weight and showed no significant difference (P>0.05) within the group at different periods of time interval.

4.1.3 Blood Urea Nitrogen

The results on blood urea nitrogen was analysed statistically and presented in Table 24 and in Fig. 5.The mean blood urea nitrogen level at 0,12,19,26 and 33rd day were 28.72±0.41, 28.78±0.45, 28.37±0.37, 28.90±0.46 and 28.77±0.43 respectively. There was no variation in the mean blood urea nitrogen level and showed no significant difference (P>0.05) within the group at different periods of time interval.

4.1.4 Microscopical Examination of Urine

Microscopical examination of urine revealed very few epithelial cells and hyaline casts at 12th and 33rd day of the experiment.

4.1.5 Gross and Histopathological Examination of Kidney

Detailed post mortem examination was conducted and the kidneys were collected for histopathology after gross examination. No characteristic gross and microscopic lesions were observed in the kidney (Fig.7.).

4.1.6 Haematology

The values on packed cell volume, haemoglobin, total erythrocyte count, total leukocyte count and differential leukocyte count were analysed statistically and presented in Table 26 and in Fig.13 to 20. All the values fell within the normal range and showed no significant difference ($P>0.05$) between the group at different period of time interval.

4.2 GENTAMICIN SULPHATE CONTROL (G2)

In this group, gentamicin sulphate was administered subcutaneously at the dose rate of 80 mg/kg body weight for 12 days continuously and produced nephrotoxicity. The values on feed intake, body weight, blood urea nitrogen and haemogram were tabulated and presented in Table 7 to 9. Microscopical examination of urine, gross and histopathological examination of kidney were also studied.

4.2.1 Feed Intake

Feed intake was tabulated, and presented in Table 3. The feed intake at 0 day was 105 gm and it was reduced to 20gm at 12^{th} day. Thus feed intake was reduced gradually towards the end of the experiment (12^{th}day) and showed significant difference within the group at different period of time interval (Fig. 3).

4.2.2 Body Weight

The results on body weight was analysed statistically and presented in Table 23 and in Fig. 4.The mean body weight at 0 and 12^{th} day was 208.75±36.82 and 184.38±39.59 respectively. There was marked reduction in the body weight and showed significant difference ($P<0.01$) within the group at different period of time interval.

4.2.3 Blood Urea Nitrogen

The results on blood urea nitrogen was analysed statistically and presented in Table 25 and in Fig.5.The mean blood urea nitrogen level at 0 and 12^{th} day were 27.33±2.051 and 287.31±9.58 respectively. The mean blood urea nitrogen level was increased markedly at 12^{th} day and showed significant difference ($P<0.01$) within the group at different periods of time interval.

4.2.4 Microscopical Examination of Urine

It revealed epithelial cells, hyaline casts, granular casts, fat globules, and urate crystals (12^{th} day).

4.2.5 Gross and Histopathological Examination of Kidney

Gross examination revealed circumscribed greyish white patches, petechiae diffusely distributed in the parenchyma. There was moderate enlargement of the kidney (Fig.6.). Degeneration of the cortical tubules, loss of lining cells, hyalinization of the tubules, occasional cystic dilatation of tubules, glomerular necrosis, medullary congestion along with focal accumulation of inflammatory cells in the cortical medullary areas were the characteristic histopathological lesions (Fig.8.).

4.2.6 Haematology

The haematological data were analysed statistically and presented in Table 26 and in Fig.13 to 20. The haemogram of the gentamicin sulphate group (12^{th} day) was compared with saline control (12^{th} day). There was significant decrease in the total leukocyte count, neutrophil count but marked increase in lymphocyte count was noticed ($P<0.01$). All other values fell within the normal range and showed no significant difference ($P>0.05$) between the group at different period of time interval.

4.3 *Aerva lanata* @ 250 mg/kg body weight (G3)

In this group, gentamicin sulphate was administered subcutaneously at the dose rate of 80 mg/kg body weight for 12 days continuously and then the extract was administered orally at the dose rate of 250 mg/kg body weight for another 21 days. The values on feed intake, body weight, blood urea nitrogen and haemogram were tabulated and presented in Table 10 to 12. Microscopical examination of urine, gross and histopathological examination of kidney were also studied.

4.3.1 Feed Intake

Feed intake was recorded, tabulated, and presented in Table 3. The feed intake at 0 day was 100 gm and it was reduced to 20gm at 12^{th} day. The decrease in feed intake was started increasing to 35gm at 13^{th} day and to 120gm at the end of the experiment (33^{rd} day) and showed significant difference within the group at different period of time intervals (Fig. 3).

4.3.2 Body Weight

The results on body weight was analysed statistically and presented in Table 22 and in Fig. 4.The mean body weight at 0,12,19,26 and 33rd day of the experiment were 211.25±5.15, 171.25±9.15, 178.75±11.09, 183.75±10.17 and 187.5±9.78 respectively. The mean body weight was reduced markedly at 12th day and then it started increasing towards the end of the experiment (33rd day) and showed significant difference ($p<0.05$) within the group at different periods of time interval.

4.3.3 Blood Urea Nitrogen

The results on mean blood urea nitrogen level was analysed statistically and presented in Table 24 and in Fig.5.The mean blood urea nitrogen level at 0,12,19,26 and 33rd day of the experiment were 28.51±0.32, 282.71±2.64, 42.57±1.16, 29.11±0.66 and 27.92±0.62 respectively. The mean blood urea nitrogen level was increased markedly at 12th day and then it started decreasing towards the end of the experiment (33rd day) and showed significant difference ($p<0.05$) within the group at different periods of time interval.

4.3.4 Microscopical Examination of Urine

Microscopical examination of urine at 12th day revealed epithelial cells, hyaline casts, granular casts, fat globules, few urate crystals but only few epithelial cells and hyaline casts were found at 33rd day of the experiment.

4.3.5 Gross and Histopathological Examination of Kidney

Kidneys on examination revealed no gross lesions. Glomerular and tubular degeneration and necrosis, interstitial infiltration with mononuclear cells and presence of hyaline casts in certain tubules were observed (Fig.9.).

4.3.6 Haematology

The haematological data were analysed statistically and presented in Table 26 and in Fig.13 to 20. The haemogram of 12^{th} day and 33^{rd} day was compared with 12^{th} and 33^{rd} day of other experimental groups respectively. There was significant reduction in the total leukocyte count and neutrophil count. But a marked increase in the lymphocyte count was noticed ($P<0.01$). All other values fell within the normal range and showed no significant difference ($P>0.05$) between the group at different period of time interval.

4.4 *Aerva lanata* @ 500 mg/kg body weight (G4)

In this group, gentamicin sulphate was administered subcutaneously at the dose rate of 80 mg/kg body weight for 12 days continuously and then the plant extract was administered orally at the dose rate of 500 mg/kg body weight for another 21 days. The values on feed intake, body weight, blood urea nitrogen and haemogram were tabulated and presented in Table 13 to 15. Microscopical examination of urine, gross and histopathological examination of kidney were also studied.

4.4.1 Feed Intake

Feed intake was recorded, tabulated, and presented in Table 3. The feed intake at 0 day was 100 gm and it was reduced to 20gm at 12^{th} day. The decrease

in feed intake was started increasing to 40gm at 13th day and to 120gm at the end of the experiment (33rd day) and showed significant difference within the group at different period of time interval (Fig. 3).

4.4.2 Body Weight

The results on body weight was analysed statistically and presented in Table 22 and in Fig. 4.The mean body weight at 0,12,19,26 and 33rd day of the experiment were 207.5±8.4, 186.25±8.22, 187.5±7.26, 205±5.35 and 211.25±4.8.The mean body weight was reduced markedly at 12th day and then it started increasing towards the end of the experiment (33rd day) and showed significant difference ($p<0.05$) within the group at different periods of time interval.

4.4.3 Blood Urea Nitrogen

The results on mean blood urea nitrogen level was analysed statistically and presented in Table 24 and in Fig.5.The mean blood urea nitrogen level at 0,12,19,26 and 33rd day of the experiment were 27.91±0.22, 284.19±2.37, 35.24±0.66, 25.37±0.63 and 25.45±0.60 respectively. The mean blood urea nitrogen level was increased markedly at 12th day and then it started decreasing towards the end of the experiment (33rd day) and showed significant difference ($p<0.05$) within the group at different periods of time interval.

4.4.4 Microscopical Examination of Urine

Microscopical examination of urine at 12th day revealed epithelial cells, hyaline casts, granular casts, fat globules, few urate crystals but only few epithelial cells and hyaline casts were found at 33rd day of the experiment.

4.4.5 Gross and Histopathological Examination of Kidney

No gross lesions were seen in the kidney. Diffuse tubular degeneration, glomerular degeneration and necrosis, occasional areas of coagulation of parenchyma, vascular sclerosis, tubular haemorrhage, and mononuclear cell infiltration were the microscopical lesions (Fig.10.).

4.4.6 Haematology

The haematological data were analysed statistically and presented in Table 26 and in Fig.13 to 20. The haemogram of 12^{th} day and 33^{rd} day was compared with 12^{th} and 33^{rd} day of other experimental groups respectively. There was significant reduction in the total leukocyte count and neutrophil count. But a marked increase in the lymphocyte count was noticed ($P<0.01$). All other values fell within the normal range and showed no significant difference ($P>0.05$) between the group at different period of time interval.

4.5 *Boerrhavia diffusa* @ 250 mg/kg body weight (G5)

In this group, gentamicin sulphate was administered subcutaneously at the dose rate of 80 mg/kg body weight for 12 days continuously and then the plant extract was administered orally at the dose rate of 250 mg/kg body weight for another 21 days The values on feed intake, body weight, blood urea nitrogen and haemogram were tabulated and presented in Table 16 to 18. Microscopical examination of urine, gross and histopathological examination of kidney were also studied.

4.5.1 Feed Intake

Feed intake was recorded, tabulated, and presented in Table 3. The feed intake at 0 day was 100 gm and it was reduced to 20gm at 12^{th} day. The decrease

in feed intake was started increasing to 30gm at 13th day and to 115gm at the end of the experiment (33rd day) and showed significant difference within the group at different period of time intervals (Fig. 3).

4.5.2 Body Weight

The results on body weight was analysed statistically and presented in Table 22 and in Fig. 4.The mean body weight at 0,12,19,26 and 33rd day of the experiment were 211.25±5.49, 186.25±4.98, 192.5±4.53, 200±3.78 and 205±2.67. The mean body weight was reduced markedly at 12th day and then it started increasing towards the end of the experiment (33rd day) and showed significant difference ($p<0.05$) within the group at different periods of time interval.

4.5.3 Blood Urea Nitrogen

The results on mean blood urea nitrogen level was analysed statistically and presented in Table 24 and in Fig.5. The mean blood urea nitrogen level at 0,12,19,26 and 33rd day of the experiment were 26.96±0.20, 292.42±4.33, 67.37±1.81, 40.22±1.32 and 29.89±0.36 respectively. The mean blood urea nitrogen level was increased markedly at 12th day and then it started decreasing towards the end of the experiment (33rd day) and showed significant difference ($p<0.05$) within the group at different periods of time interval.

4.5.4 Microscopical Examination of Urine

Microscopical examination of urine at 12th day revealed epithelial cells, hyaline casts, granular casts, fat globules, few urate crystals but only few epithelial cells and hyaline casts were found at 33rd day of the experiment.

4.5.5 Gross and Histopathological Examination of Kidney

Kidneys on examination revealed no gross lesions. Diffused glomerular necrosis and hypocellularity were seen (Fig.11.).

4.5.6 Haematology

The haematological data were analysed statistically and presented in Table 26 and in Fig.13 to 20. The haemogram of 12^{th} day and 33^{rd} day was compared with 12^{th} and 33^{rd} day of other experimental groups respectively. There was significant reduction in the total leukocyte count and neutrophil count. But a marked increase in the lymphocyte count was noticed ($P<0.01$). All other values fell within the normal range and showed no significant difference ($P>0.05$) between the group at different period of time interval.

4.6 *Boerrhavia diffusa* @ 500 mg/kg body weight (G6)

In this group, gentamicin sulphate was administered subcutaneously at the dose rate of 80 mg/kg body weight for 12 days continuously and then the plant extract was administered orally at the dose rate of 500 mg/kg body weight for another 21 days. The values on feed intake, body weight, blood urea nitrogen and haemogram were tabulated and presented in Table 19 to 21. Microscopical examination of urine, gross and histopathological examination of kidney were also studied.

4.6.1 Feed Intake

Feed intake was recorded, tabulated, and presented in Table 3. The feed intake at 0 day was 100 gm and it was reduced to 20gm at 12th day. The decrease in feed intake was started increasing to 30gm at 13th day and to 120gm at the end of the experiment (33rd day) and showed significant difference within the group at different period of time intervals (Fig.3).

4.6.2 Body Weight

The results on body weight was analysed statistically and presented in Table 22 and in Fig. 4. The mean body weight at 0,12,19,26 and 33rd day of the experiment were 227.5±7.0, 201.25±2.95, 202.5±3.13, 205±2.67 and 208.75±2.27.The mean body weight was reduced markedly at 12th day and then it started increasing towards the end of the experiment (33rd day) and showed significant difference ($p<0.05$) within the group at different periods of time interval.

4.6.3 Blood Urea Nitrogen

The results on mean blood urea nitrogen level was analysed statistically and presented in Table 24 and in Fig.5. The mean blood urea nitrogen level at 0,12,19,26 and 33rd day of the experiment were 27.71±0.17, 292.07±3.90, 48.88±0.72, 32.30±0.70 and 28.65±0.43 respectively. The mean blood urea nitrogen level was increased markedly at 12th day and then it started decreasing towards the end of the experiment (33rd day) and showed significant difference ($p<0.05$) within the group at different periods of time interval.

4.6.4 Microscopical Examination of Urine

Microscopical examination of urine at 12th day revealed epithelial cells, hyaline casts, granular casts, fat globules, few urate crystals but only few epithelial cells and hyaline casts were found at 33rd day of the experiment.

4.6.5 Gross and Histopathological Examination of Kidney

Kidneys on examination revealed no gross lesions. Histopathological examination revealed diffuse degeneration and necrosis of tubules. In focal areas, the tubules appeared bound by more than one layer of cells. Mononuclear infiltration could also be seen (Fig.12.).

4.6.6 Haematology

The haematological data were analysed statistically and presented in Table 26 and in Fig.13 to 20. The haemogram of 12th day and 33rd day was compared with 12th and 33rd day of other experimental groups respectively. There was significant reduction in the total leukocyte count and neutrophil count. But a marked increase in the lymphocyte count was noticed ($P<0.01$). All other values fell within the normal range and showed no significant difference ($P>0.05$) between the group at different period of time interval.

Table 3. Control and Test drug group.
Feed intake (g)

Day	G_1	G_2	G_3	G_4	G_5	G_6
0	104	105	100	100	100	100
1	112	75	95	80	80	90
2	108	75	84	80	80	80
3	112	65	71	75	75	75
4	116	60	60	75	65	70
5	116	50	60	70	60	65
6	112	40	50	50	60	60
7	120	40	40	40	55	55
8	120	40	40	40	50	50
9	124	30	30	40	45	40
10	120	20	30	30	35	40
11	120	20	20	30	30	30
12	110	sacrificed	20	20	20	20
13	116		35	40	30	30
14	120		40	50	40	35
15	130		45	50	40	40
16	130		50	60	50	50
17	125		50	70	60	65
18	130		90	100	80	75
19	110		60	75	60	60
20	120		60	80	80	80
21	120		80	80	90	80
22	120		100	80	90	95
23	115		100	80	90	100
24	115		100	90	100	110
25	120		100	95	110	110
26	110		80	60	90	80
27	120		100	80	100	100
28	120		120	100	100	110
29	115		120	110	100	110
30	115		120	110	110	115
31	120		115	115	115	115
32	120		120	120	115	120

G1-Saline Control,
G2-Gentamicin Sulphate Control,
G3- *Aerva lanata* @ 250 mg/kg
G4- *Aerva lanata* @ 500 mg/kg,
G5- *Boerrhavia diffusa* @ 250 mg/kg,
G6- *Boerrhavia diffusa* @ 500 mg/kg.

Table 4. Saline control group (G1)
Body weight (g)

Animal No.	Day				
	0	12	19	26	33
1	190	195	195	200	200
2	220	220	225	225	225
3	210	210	210	210	210
4	190	190	190	195	195
5	200	205	200	200	200
6	200	200	200	200	200
7	160	165	165	170	170
8	220	220	220	220	220

G1-Group 1

Table 5. Saline control group (G1)
Blood urea nitrogen (mg/dl)

Animal No.	Day				
	0	12	19	26	33
1	28.61	27.86	28.61	28.88	29.83
2	30.20	30.95	30.20	30.73	30.20
3	27.33	28.48	27.11	27.68	27.33
4	27.69	27.72	27.47	27.52	27.25
5	28.41	28.65	28.21	28.42	28.20
6	29.13	28.79	28.12	29.99	29.79
7	30.53	30.34	29.75	30.34	29.67
8	27.89	27.41	27.67	27.61	27.89

G1-Group 1

Table 6. Saline control group (G_1)
Haematology

Animal No.	PCV(%)		Hb(g %)		TEC(10^6/mm^3)		TLC(10^3/mm^3)		DLC(%)							
									N		E		L		M	
	12	33	12	33	12	33	12	33	12	33	12	33	12	33	12	33
1	46	47	14.6	13.8	8.29	8.72	7,800	8,760	24	28	3	2	73	70	0	0
2	47	48	14.8	14.6	8.58	8.93	8,100	9,240	26	24	1	2	72	74	1	0
3	48	45	15.2	15.0	8.22	8.57	8,200	8,120	23	25	3	1	74	73	0	1
4	44	45	15.0	15.2	8.81	8.07	9,350	8,830	27	22	1	2	71	76	1	0
5	46	45	15.8	15.6	8.02	8.30	9,100	8,130	21	28	2	2	77	70	0	0
6	45	46	14.2	14.4	8.22	8.20	8,230	7,150	28	25	2	2	70	73	0	0
7	48	48	14.6	14.8	8.60	8.70	8,300	7,960	25	24	3	2	72	74	0	0
8	47	48	14.0	14.2	8.60	8.85	9,600	9,120	21	22	3	3	76	75	0	0

12 - 12th day
33 – 33rd day

PCV-Packed Cell Volume
Hb -Haemoglobin
TEC-Total Erythrocyte Count
TLC- Total Leukocyte Count
DLC-Differential Leukocyte Count
N-Neutrophil, E-Eosinophil, L-Lymphocyte, M-Monocyte.

Table 7. Gentamicin sulphate control (G2)
Body weight (g)

Animal No.	Day	
	0	12
1	140	110
2	180	170
3	260	240
4	200	165
5	210	170
6	220	210
7	240	200
8	220	210

G2-Group 2

Table 8. Gentamicin sulphate control (G2)
Blood urea nitrogen (mg/dl)

Animal No.	Day	
	0	12
1	24.82	297.83
2	24.46	275.54
3	26.97	297.52
4	28.05	294.74
5	29.49	278.64
6	29.85	281.73
7	26.25	278.32
8	28.77	294.12

G2-Group 2

Table 9. Gentamicin sulphate control (G2)-12th Day
Haematology

Animal No.	PCV (%)	Hb (g %)	TEC (10^6/mm^3)	TLC (10^3/mm^3)	DLC(%)			
					N	E	L	M
1	45	14.6	8.27	5,900	17	3	79	1
2	44	14.4	8.12	6,500	19	3	78	0
3	47	15.4	8.14	5,500	20	1	79	0
4	48	15.6	8.93	6,000	20	0	80	0
5	46	14.6	8.01	7,100	18	3	79	0
6	47	14.8	8.91	5,600	17	3	80	0
7	48	14.6	8.76	6,800	17	3	80	0
8	46	14.8	8.93	7,150	19	1	80	0

G2-Group 2

Table 10. Test drug group (G3) – ***Aerva lanata*** **@ 250 mg/kg**
Body weight (g)

Animal No.	Day				
	0	12	19	26	33
1	200	160	170	170	170
2	200	180	180	180	190
3	210	180	180	190	190
4	220	180	180	180	180
5	200	140	150	160	160
6	220	140	150	160	170
7	240	220	250	250	250
8	200	170	170	180	190

G3-Group 3

Table 11. Test drug group (G3) – ***Aerva lanata*** **@ 250 mg/kg**
Blood urea nitrogen (mg/dl)

Animal No.	Day				
	0	12	19	26	33
1	28.51	277.08	41.07	30.83	29.72
2	29.12	278.63	37.76	29.20	28.10
3	27.23	296.28	41.36	28.43	27.33
4	27.59	282.35	41.72	28.35	27.24
5	28.52	281.42	40.04	25.21	24.20
6	28.12	292.10	45.08	31.22	29.12
7	30.12	276.30	47.12	29.67	28.52
8	28.86	277.52	46.40	29.96	29.12

G3-Group 3

Table 12. Test drug group (G_3) – ***Aerva lanata* @ 250 mg/kg**
Haematology

Animal No.	PCV(%)		Hb(g %)		TEC(10^6/mm^3)		TLC(10^3/mm^3)		DLC(%)							
									N		E		L		M	
	12	33	12	33	12	33	12	33	12	33	12	33	12	33	12	33
1	43	48	13.76	14.00	8.29	8.69	5,600	8416	18	23	2	2	80	75	0	0
2	42	47	14.30	14.20	8.72	8.32	6,300	8372	20	23	1	3	78	74	1	0
3	47	45	15.08	14.98	8.43	8.29	5,250	8816	17	26	3	2	80	72	0	0
4	48	46	14.64	15.00	8.65	8.10	6,150	8832	20	27	1	2	79	71	0	0
5	48	46	14.00	14.62	8.41	8.04	6,220	8444	17	23	3	1	80	75	0	1
6	47	44	14.24	14.34	8.56	8.78	5,750	8110	19	24	2	2	79	74	0	0
7	46	48	14.24	14.44	8.17	8.71	6,220	8430	19	25	2	3	79	72	0	0
8	45	47	14.67	14.00	8.34	8.14	7,150	8120	17	27	3	1	80	72	0	0

12 - 12th day
33 – 33rd day

PCV-Packed Cell Volume
Hb -Haemoglobin
TEC-Total Erythrocyte Count
TLC- Total Leukocyte Count
DLC-Differential Leukocyte Count
N-Neutrophil, E-Eosinophil, L-Lymphocyte, M-Monocyte.

Table 13. Test drug group (G4) – *Aerva lanata* @ 500 mg/kg
Body weight (g)

Animal No.	Day				
	0	12	19	26	33
1	180	160	160	200	200
2	200	180	200	210	210
3	200	180	200	200	220
4	200	180	190	200	210
5	200	180	190	200	200
6	260	240	220	240	240
7	220	190	160	190	200
8	200	180	180	200	210

G4-Group 4

Table 14 Test drug group (G4) – *Aerva lanata* @ 500 mg/kg
Blood urea nitrogen (mg/dl)

Animal No.	Day				
	0	12	19	26	33
1	27.28	279.04	35.86	26.41	25.14
2	27.64	280.85	33.33	28.01	27.10
3	28.79	294.06	34.78	23.11	22.11
4	28.50	284.57	34.66	27.25	26.25
5	27.49	283.64	34.60	24.11	25.14
6	28.58	275.54	34.05	25.12	27.12
7	27.25	294.12	39.49	25.52	26.52
8	27.77	281.73	35.14	23.45	24.25

G4-Group 4

Table 15. Test drug group (G_4) – ***Aerva lanata*** **@ 500 mg/kg**
Haematology

Animal No.	PCV(%)		Hb(g %)		TEC(10^6/mm^3)		TLC(10^3/mm^3)		DLC(%)							
									N		E		L		M	
	12	33	12	33	12	33	12	33	12	33	12	33	12	33	12	33
1	45	45	14.33	14.00	8.99	8.26	5750	8596	18	27	2	1	80	71	0	1
2	46	47	14.40	14.00	8.83	8.89	6150	8682	17	26	3	2	79	72	1	0
3	48	46	14.92	15.00	8.31	8.21	5700	8532	17	23	3	2	80	75	0	0
4	47	48	14.84	14.76	8.28	8.68	6550	8664	19	24	2	3	79	73	0	0
5	47	46	14.83	14.70	8.04	8.12	6720	8504	19	26	2	2	79	71	0	1
6	44	45	15.00	15.00	8.93	8.62	5920	8696	21	27	1	2	78	71	0	0
7	44	44	14.62	14.72	8.39	8.49	6520	8516	20	22	2	3	78	75	0	0
8	46	47	14.56	14.82	8.46	8.36	6650	8216	18	23	2	2	80	75	0	0

12 - 12th day
33 – 33rd day

PCV-Packed Cell Volume
Hb -Haemoglobin
TEC-Total Erythrocyte Count
TLC- Total Leukocyte Count
DLC-Differential Leukocyte Count
N-Neutrophil, E-Eosinophil, L-Lymphocyte, M-Monocyte.

Table 16. Test drug group (G5) –***Boerrhavia diffusa*** **@ 250 mg/kg**
Body weight (g)

Animal No.	Day				
	0	12	19	26	33
1	210	200	210	210	210
2	200	170	180	190	200
3	210	190	180	190	200
4	200	170	180	200	200
5	240	200	210	220	220
6	200	190	190	200	200
7	200	170	190	190	200
8	230	200	200	200	210

G5-Group 5

Table 17. Test drug group (G5) –***Boerrhavia diffusa*** **@ 250 mg/kg**
Blood urea nitrogen (mg/dl)

Animal No.	Day				
	0	12	19	26	33
1	28.16	295.63	65.10	42.72	30.88
2	27.02	279.32	68.71	34.98	30.62
3	26.33	295.66	61.51	39.62	29.66
4	26.69	309.28	71.94	38.85	28.62
5	27.14	302.79	75.89	37.30	29.53
6	27.13	299.69	64.38	39.10	29.69
7	27.35	274.92	69.85	41.17	31.44
8	27.89	282.04	61.58	42.00	28.65

G5-Group 5

Table 18. Test drug group (G_5) – ***Boerrhavia diffusa*** **@ 250 mg/kg**
Haematology

Animal No.	PCV(%)		Hb(g %)		TEC(10^6/mm^3)		TLC(10^3/mm^3)		DLC(%)							
									N		E		L		M	
	12	33	12	33	12	33	12	33	12	33	12	33	12	33	12	33
1	47	47	14.60	15.00	8.20	8.12	5920	8126	17	22	3	3	80	75	0	0
2	48	46	14.84	14.72	8.10	8.32	6850	8182	17	24	3	2	79	73	1	1
3	45	47	14.10	14.70	8.70	8.50	6660	8432	18	26	2	1	80	73	0	0
4	45	45	15.52	15.00	8.95	8.60	6450	8632	20	24	1	3	79	73	0	0
5	44	47	14.56	14.84	8.92	8.29	6520	8200	18	23	3	2	79	75	0	0
6	45	44	14.80	14.92	8.93	8.68	6650	8300	19	27	2	2	79	71	0	0
7	44	43	14.12	14.64	8.37	8.12	6420	8230	20	25	1	2	79	73	0	0
8	44	44	14.24	14.34	8.17	8.71	7050	8220	19	26	2	2	79	72	0	0

12 - 12th day
33 – 33rd day

PCV-Packed Cell Volume
Hb -Haemoglobin
TEC-Total Erythrocyte Count
TLC- Total Leukocyte Count
DLC-Differential Leukocyte Count
N-Neutrophil, E-Eosinophil, L-Lymphocyte, M-Monocyte.

Table 19. Test drug group (G6) –***Boerrhavia diffusa*** **@ 500 mg/kg**
Body weight (g)

Animal No.	Day				
	0	12	19	26	33
1	240	200	200	200	210
2	240	200	200	200	210
3	250	210	200	200	210
4	250	210	220	220	220
5	210	200	200	200	200
6	200	190	200	210	210
7	210	190	190	200	200
8	220	210	210	210	210

G6-Group 6

Table 20.Test drug group (G6) –***Boerrhavia diffusa*** **@ 500 mg/kg**
Blood urea nitrogen (mg/dl)

Animal No.	Day				
	0	12	19	26	33
1	28.28	295.66	48.92	30.25	27.25
2	27.46	292.26	46.76	30.81	28.53
3	27.79	276.16	47.84	34.53	26.72
4	27.05	278.95	47.84	34.64	30.54
5	28.49	288.85	48.38	32.89	28.79
6	27.58	292.26	47.30	30.97	28.77
7	27.25	305.88	52.50	30.13	29.31
8	27.77	306.54	51.43	34.17	29.25

G6-Group 6

Table 21. Test drug group (G6) – ***Boerrhavia diffusa*** **@ 500 mg/kg**
Haematology

Animal No.	PCV(%)		Hb(g %)		TEC(10^6/mm^3)		TLC(10^3/mm^3)		DLC(%)							
									N		E		L		M	
	12	33	12	33	12	33	12	33	12	33	12	33	12	33	12	33
1	48	47	15.2	15.0	8.83	8.32	5,660	8,120	20	25	1	2	79	73	0	0
2	46	47	14.4	14.6	8.67	8.20	6,350	8,320	19	24	2	2	79	74	0	0
3	46	48	14.8	14.4	8.68	8.52	5,800	8,140	19	21	2	3	79	75	0	1
4	45	46	14.6	14.4	8.79	8.19	6,750	8,160	16	26	3	2	80	72	1	0
5	47	45	14.2	14.6	8.09	8.59	6,800	8,500	18	23	3	3	79	74	0	0
6	45	47	14.2	14.6	8.72	8.88	6,620	8,596	17	25	3	2	80	73	0	0
7	46	45	14.4	15.0	8.10	8.57	6,720	8,216	18	24	2	2	80	74	0	0
8	46	45	14.6	14.0	8.52	8.62	6,520	8,916	20	27	1	1	79	72	0	0

12 - 12th day
33 – 33rdday

PCV-Packed Cell Volume
Hb -Haemoglobin
TEC-Total Erythrocyte Count
TLC- Total Leukocyte Count
DLC-Differential Leukocyte Count
N-Neutrophil, E-Eosinophil, L-Lymphocyte, M-Monocyte.

Table 22. Summary of observations – Body weight (g)
Analysis of variance table:

Source	Degrees of freedom	Sum of squares	Mean square	F value
Factor A	4	10440.500	2610.125	1.8295
Error	35	49934.375	1426.696	
Factor B	4	12203.000	3050.750	45.4972*
AB	16	6419.500	401.219	5.9836*
Error	140	9387.500	67.054	

P<0.01 * Significant at 1 % level

Group	0 day Mean ± SE	12 day Mean ± SE	19 day Mean ± SE	26 day Mean ± SE	33 day Mean ± SE
G_1	A 198.750 ± 6.93 b	A 201.250 ± 6.67 a	A 200.63 ± 6.64 a	A 202.500 ± 5.98 a	A 202.500 ± 5.98 ab
G_3	A 211.250 ± 5.15 ab	D 171.250 ± 9.15 b	CD 178.750 ± 11.09 b	BC 183.750 ± 10.17 b	B 187.500 ± 9.78 b
G_4	A 207.500 ± 8.4 b	B 186.250 ± 8.22 ab	B 187.500 ± 7.26 ab	A 205.000 ± 5.35 a	A 211.250 ± 4.8 a
G_5	A 211.250 ± 5.49 ab	D 186.250 ± 4.98 ab	CD 192.500 ± 4.52 ab	BC 200.000 ± 3.78 ab	AB 205.000 ± 2.67 ab
G_6	A 227.500 ± 7.0 a	B 201.250 ± 2.95 a	B 202.500 ± 3.13 a	B 205.000 ± 2.67 a	B 208.750 ± 2.27 a

P<0.05 Small letters – Comparison between groups
Capital letters – Comparison within groups
Treatments having common letters are homogenous.

Table 23:Gentamicin sulphate control (G2)- Body weight (g)
Students- 't' test (0 day with 12th day)

Day	Mean	SD	t
0	208.75	36.82	5.1147 *
12	184.38	39.59	

P<0.01 * Significant at 1%level

Table 24. Summary of observations – Blood urea nitrogen (mg/dl)

Analysis of variance table:

Source	Degrees of freedom	Sum of squares	Mean square	F value
Factor A	4	103706.283	25926.571	1366.9627*
Error	35	663.829	18.967	
Factor B	4	1327264.677	331816.169	18242.8044*
AB	16	334726.283	20920.393	1150.1749*
Error	140	2546.443	18.189	

P<0.01 * Significant at 1 % level

Group	0 day Mean ± SE	12 day Mean ± SE	19 day Mean ± SE	26 day Mean ± SE	33 day Mean ± SE
G_1	A 28.724 ± 0.410 a	A 28.775 ± 0.445 d	A 28.370 ± 0.370 e	A 28.896 ± 0.461 bc	A 28.770 ± 0.433 a
G_3	C 28.509 ± 0.320 a	A 282.710 ± 2.641 a	B 42.569 ± 1.162 c	C 29.109 ± 0.664 bc	C 27.919 ± 0.615 a
G_4	C 27.913 ± 0.218 a	A 284.194 ± 2.372 c	B 35.239 ± 0.661 d	C 25.373 ± 0.628 c	C 25.454 ± 0.599 a
G_5	D 26.964 ± 0.198 a	A 292.417 ± 4.332 b	B 67.370 ± 1.810 a	C 40.217 ± 1.317 a	D 29.886 ± 0.361 a
G_6	D 27.709 ± 0.173 a	A 292.070 ± 3.893 b	B 48.871 ± 0.720 b	C 32.299 ± 0.697 b	CD 28.645 ± 0.425 a

P<0.05 Small letters – Comparison between groups

Capital letters – Comparison within groups

Treatments having common letters are homogenous.

Table 25. Gentamicin sulphate control (G2) – Blood urea nitrogen (mg/dl)

Students -'t' test (0 day with 12th day)

Day	Mean	SD	t
0	27.332	2.051	75.07 *
12	287.305	9.578	

P < 0.01 * Significant at 1% level

Table 26. Summary – Comparison of haematology of experimental groups at 12th and 33rd day. (Mean ± SD)

	12th day						33rd day				
	G1	G2	G3	G4	G5	G6	G1	G3	G4	G5	G6
PCV (%)	46.38 ±1.41	46.38 ±1.41	45.75 ±2.25	45.88 ±1.46	45.25 ±1.49	46.13 ±0.99	46.5 ±1.41	46.38 ±1.41	46.0 ±1.31	45.38 ±1.6	46.25 ±1.17
Hb (g %)	14.78 ±0.57	14.85 ±0.42	14.36 ±0.42	14.69 ±0.25	14.6 ±0.47	14.55 ±0.33	14.7 ±0.58	14.45 ±0.40	14.63 ±0.4	14.77 ±0.22	14.58 ±0.33
TEC (10^6/ mm^3)	8.42 ±0.27	8.51± 0.41	8.45 ±0.19	8.53± 0.35	8.54 ±0.37	8.55 ±0.30	8.54 ±0.37	8.38 ±0.30	8.45 ±0.26	8.42 ±0.24	8.49 ±0.24
TLC (10^3/ mm^3)	8585± 664.14	6318.75* ±657.89	6080*± 568.91	6248.75* ±421.61	6565*± 334.88	6402.5* ±440.41	8413.75 ±701.47	8442.5± 270.35	8550.75 ±155.38	8290.25 ±165.78	8371± 280.76
N (%)	24.38 ±2.62	18.38* ±1.30	18.38* ±1.30	18.63* ±1.41	18.5* ±1.2	18.38* ±1.41	24.75 ±2.31	24.75 ±1.75	24.75 ±1.98	24.63 ±1.69	24.38 ±1.85
E (%)	2.25± 0.89	2.13 ±1.25	2.13 ±0.83	2.13 ±0.64	2.13 ±0.83	2.13 ±0.83	2.0 ±0.53	2.0 ±0.76	2.13 ±0.64	2.13 ±0.64	2.13 ±0.64
L (%)	73.13 ±2.42	79.38* ±0.74	79.38* ±0.74	79.13* ±0.83	79.25* ±0.46	79.38* ±0.52	73.13 ±2.17	73.13 ±1.55	72.88 ±1.89	73.13 ±1.36	73.38 ±1.06
M (%)	0.25± 0.46	0.125 ±0.35	0.125 ±0.35	0.125 ±0.35	0.125 ±0.35	0.125 ±0.35	0.125 ±0.35	0.125 ±0.35	0.250 ±0.46	0.125 ±0.35	0.125 ±0.35

($P<0.01$) * Significant at 1 % level

G1-Saline Control, G2-Gentamicin Sulphate Control, G3- *Aerva lanata* @ 250 mg/kg

G4- *Aerva lanata* @ 500 mg/kg, G5- *Boerrhavia diffusa* @ 250 mg/kg, G6- *Boerrhavia diffusa* @ 500 mg/kg.

PCV-Packed Cell Volume, Hb -Haemoglobin, TEC-Total Erythrocyte Count TLC- Total Leukocyte Count

DLC-Differential Leukocyte Count: N-Neutrophil, E-Eosinophil, L-Lymphocyte, M-Monocyte.

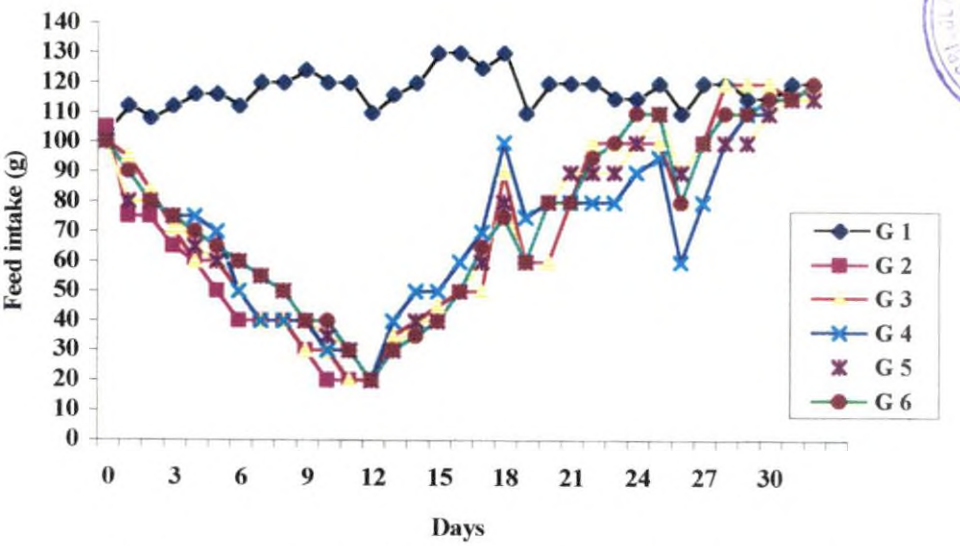


Fig. 3. Feed intake of experimental groups maintained on different treatments

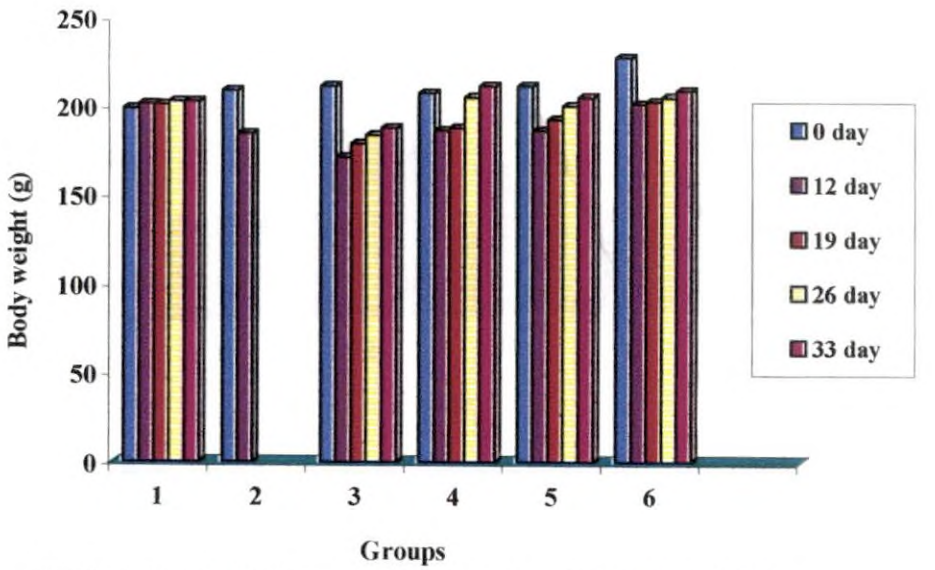
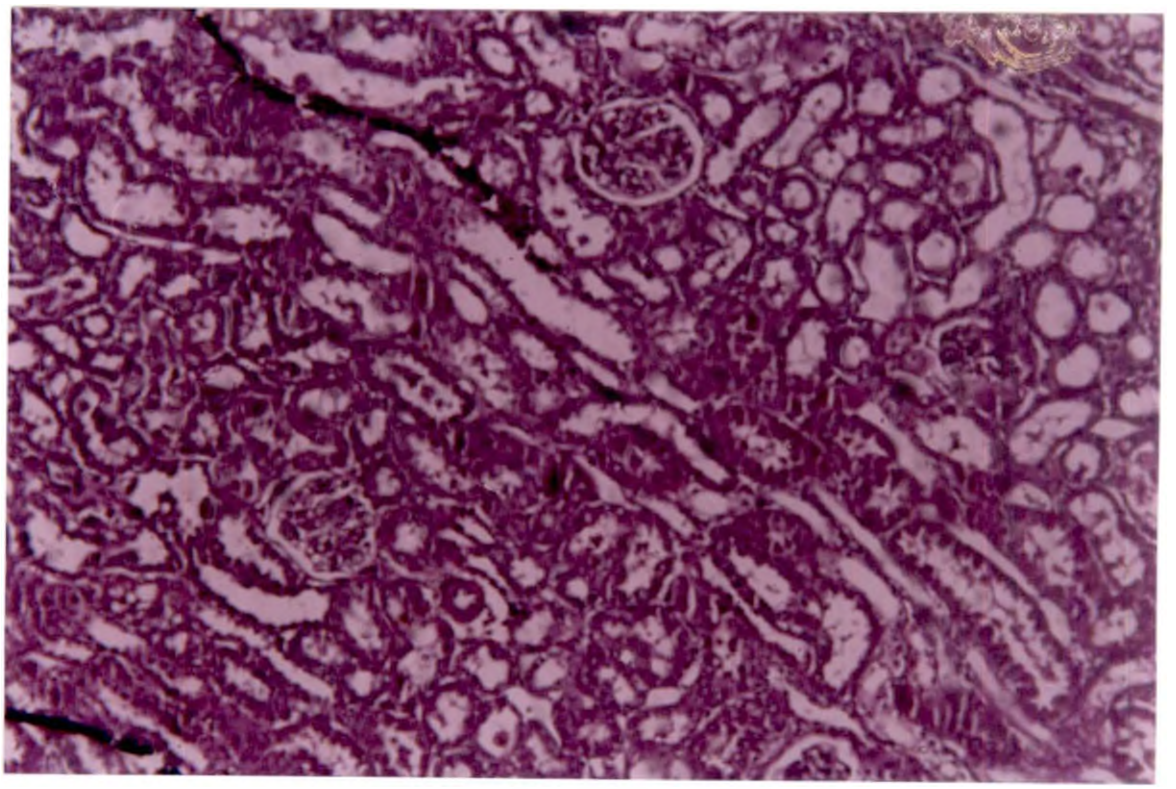
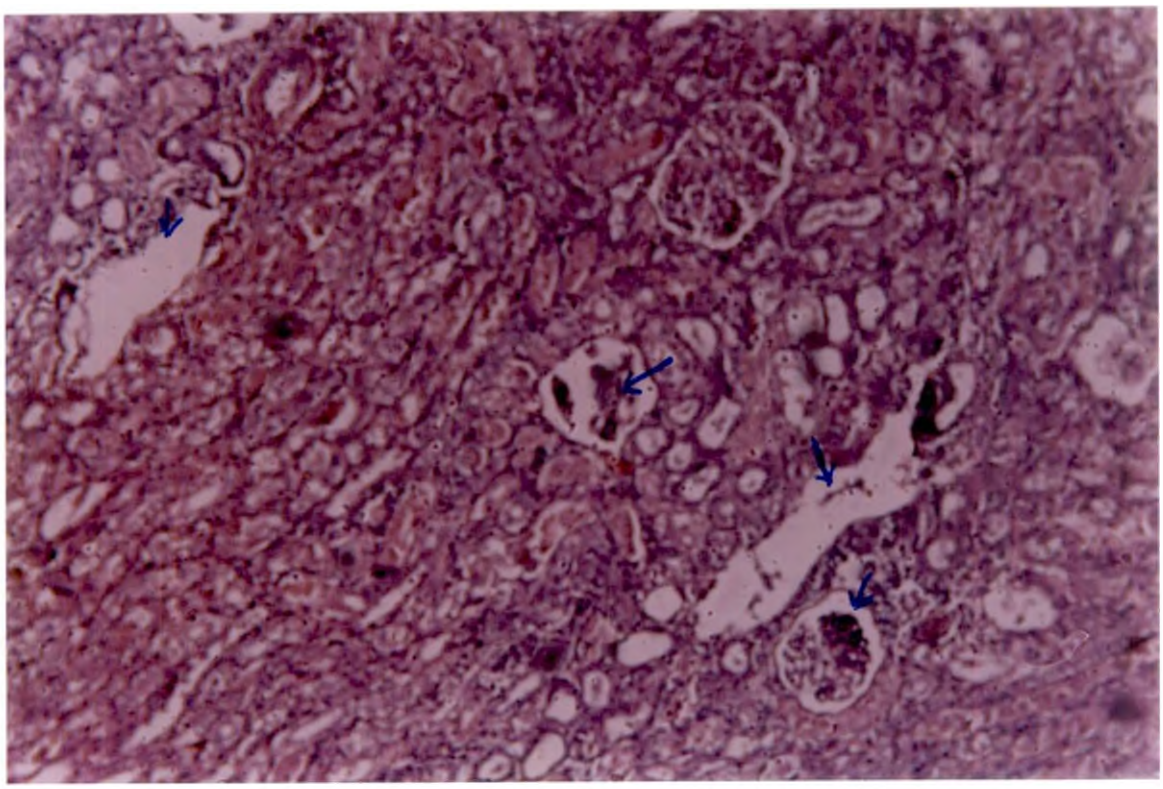
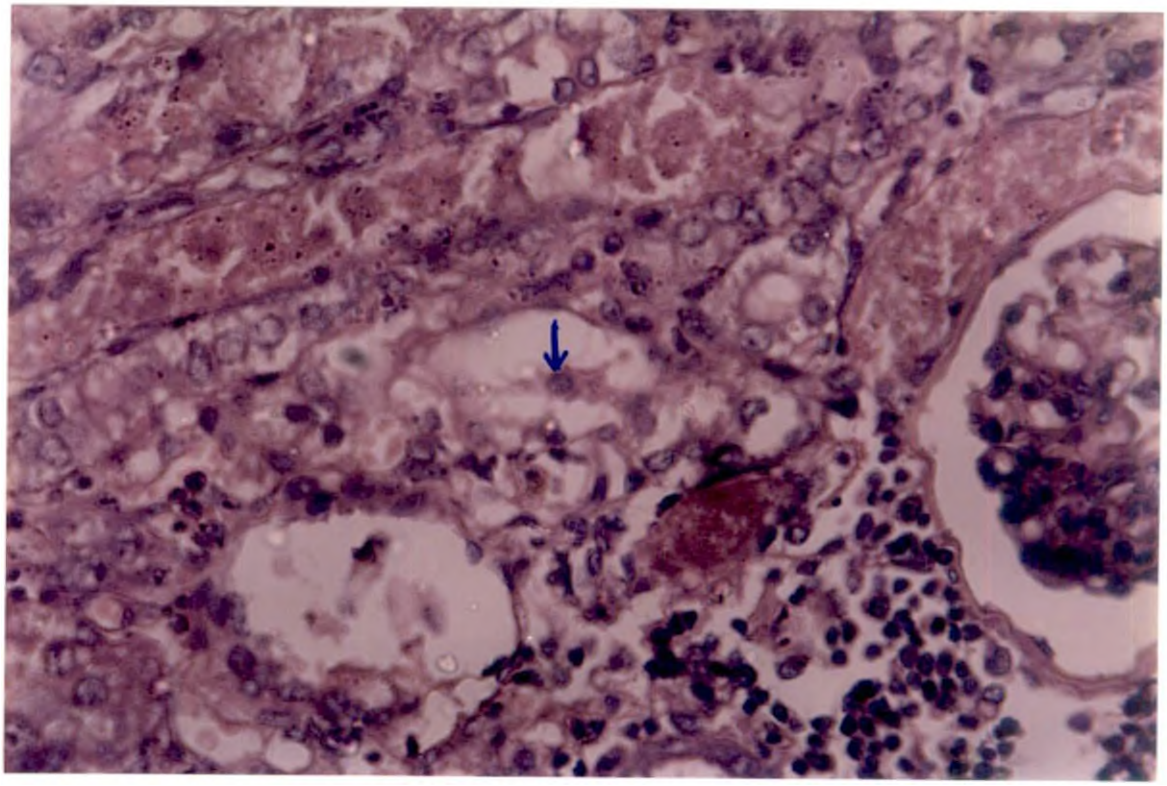


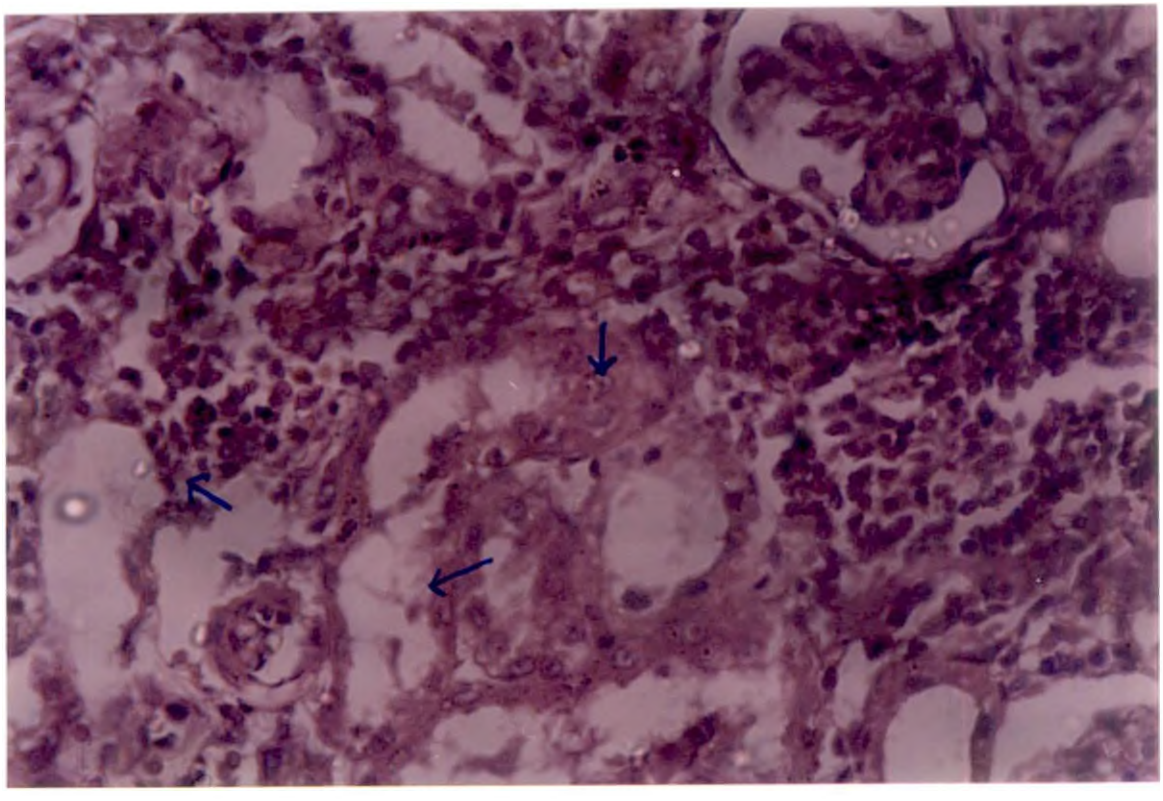
Fig.4. Body weight of experimental groups maintained on different treatments

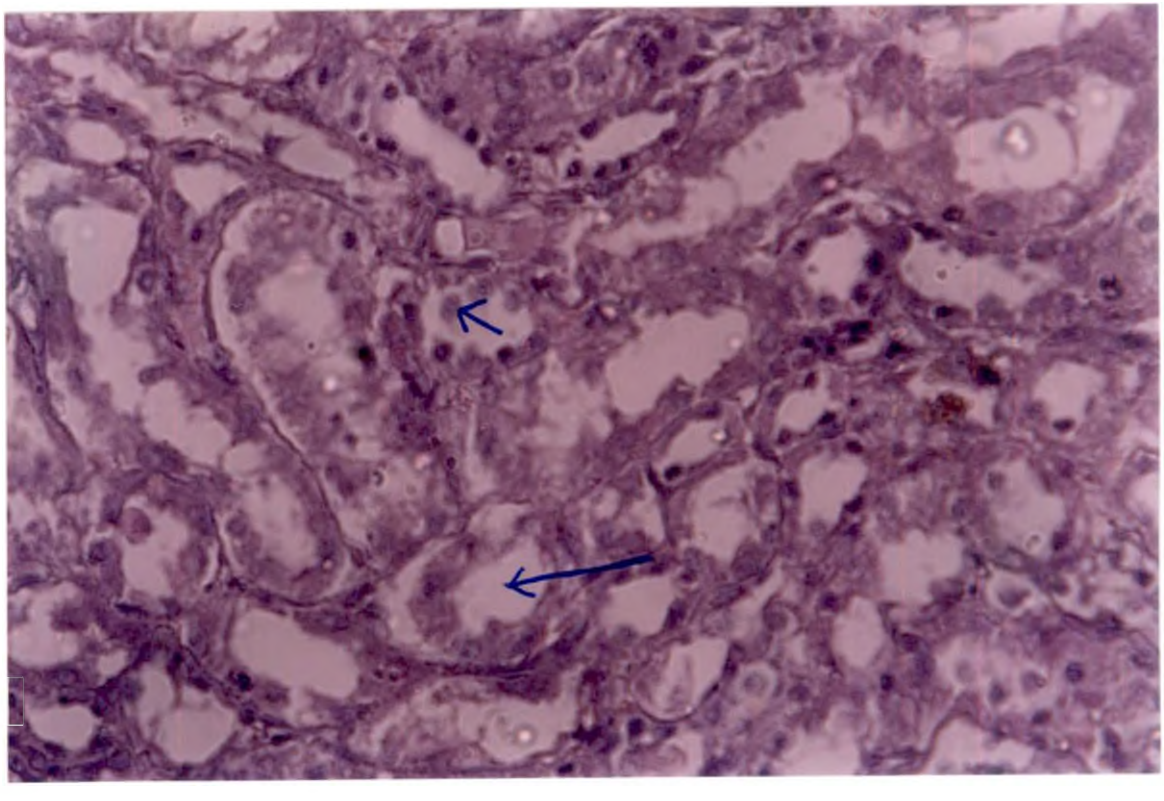

Normal
Gentamicin

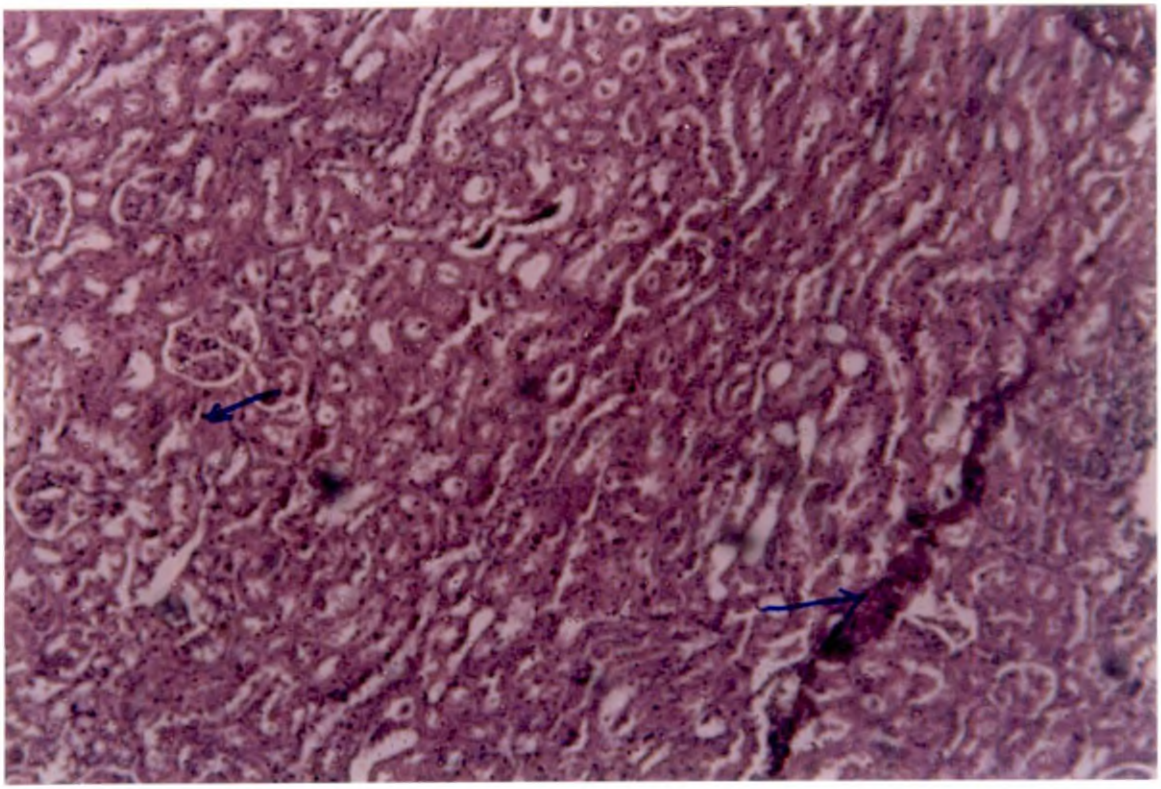


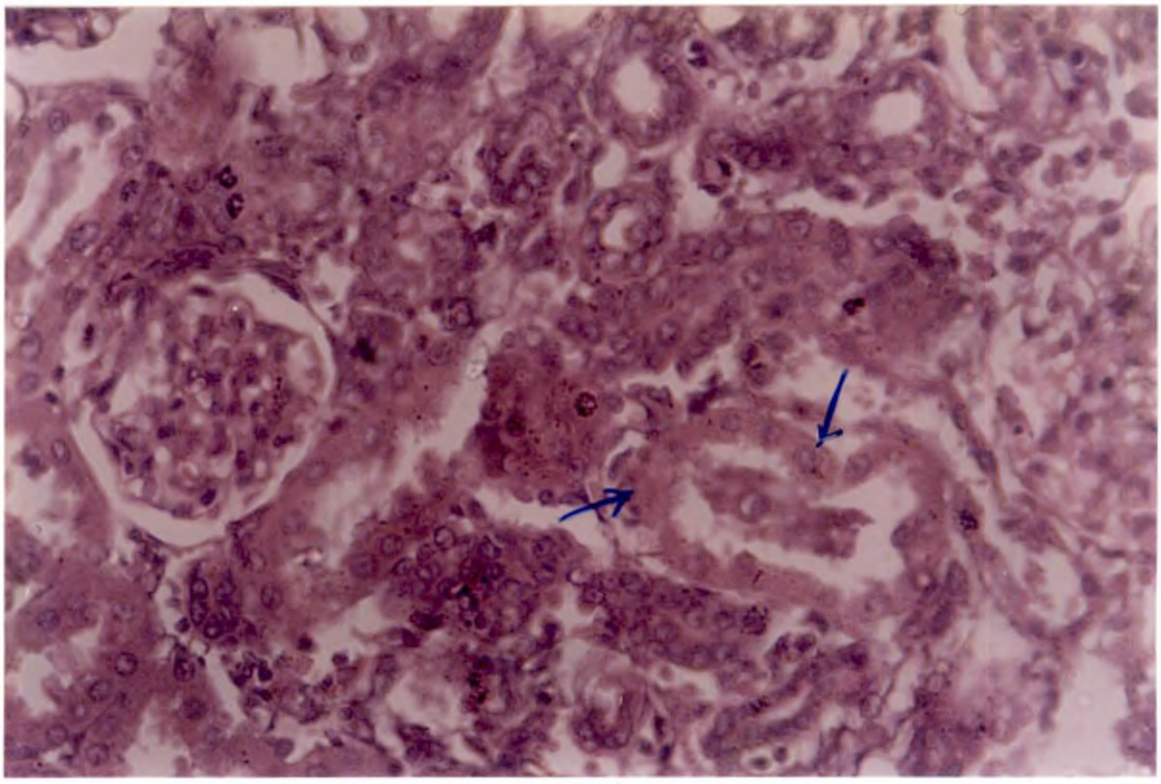












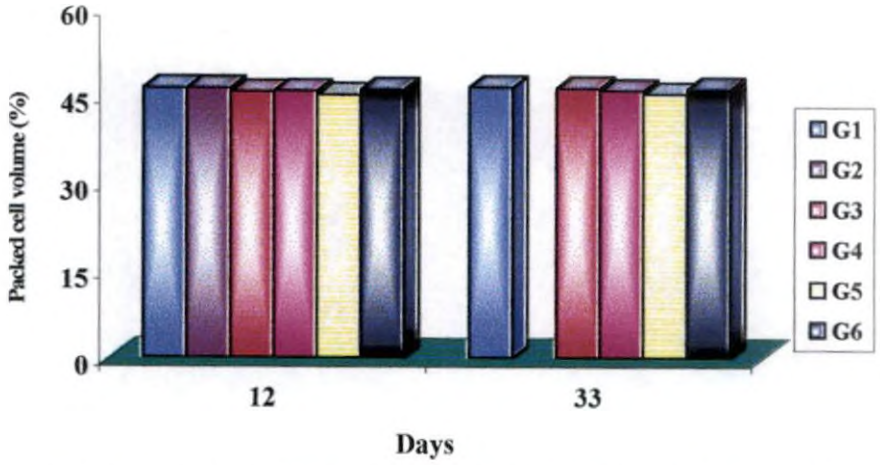


Fig.13. Packed cell volume of experimental groups maintained on different treatments

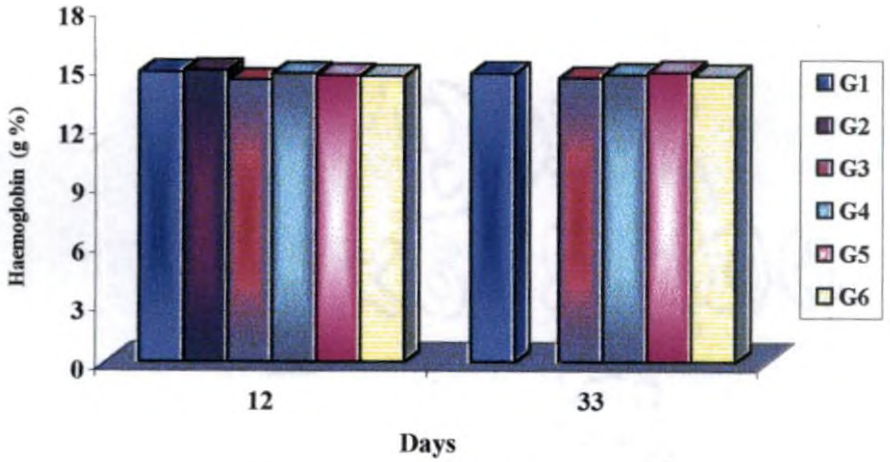


Fig.14.Haemoglobin concentration of experimental groups maintained on different treatments

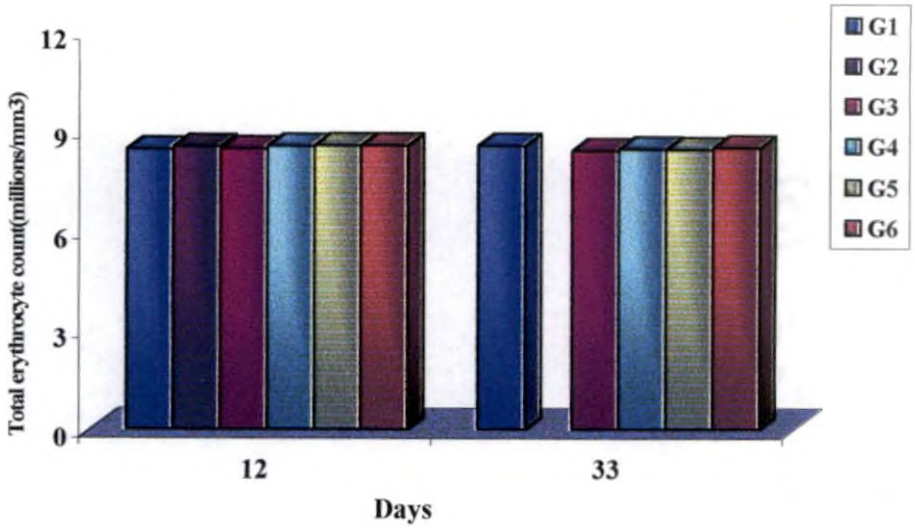


Fig.15.Total erythrocyte count of experimental groups maintained on different treatments

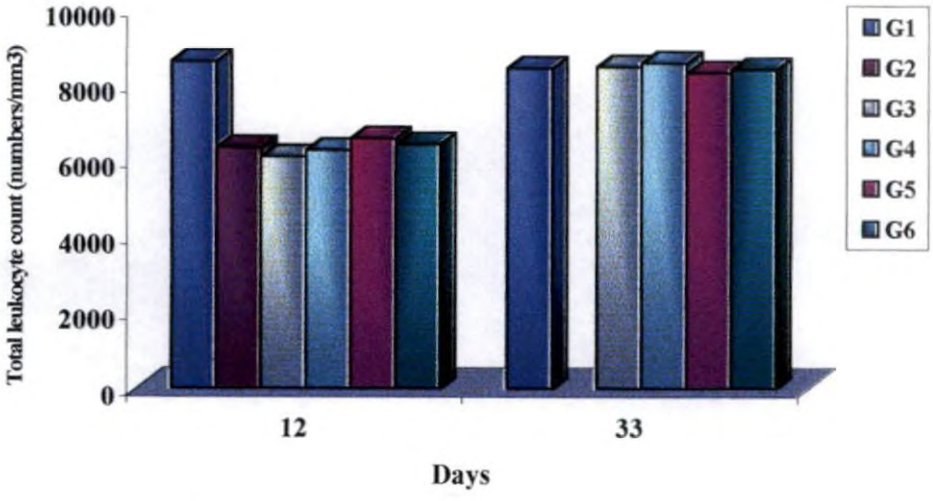


Fig.16. Total leukocyte count of experimental groups maintained on different treatments

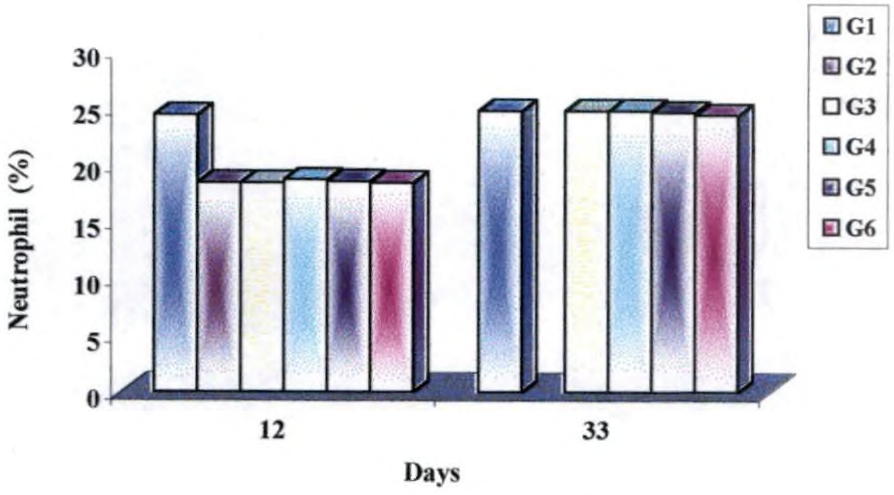


Fig. 17. Neutrophil count of experimental groups maintained on different treatments

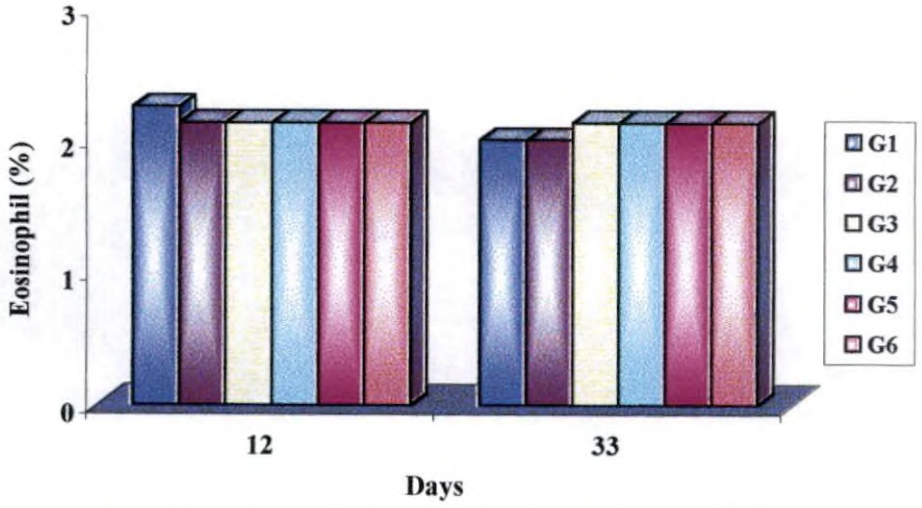


Fig.18. Eosinophil count of experimental groups maintained on different treatments

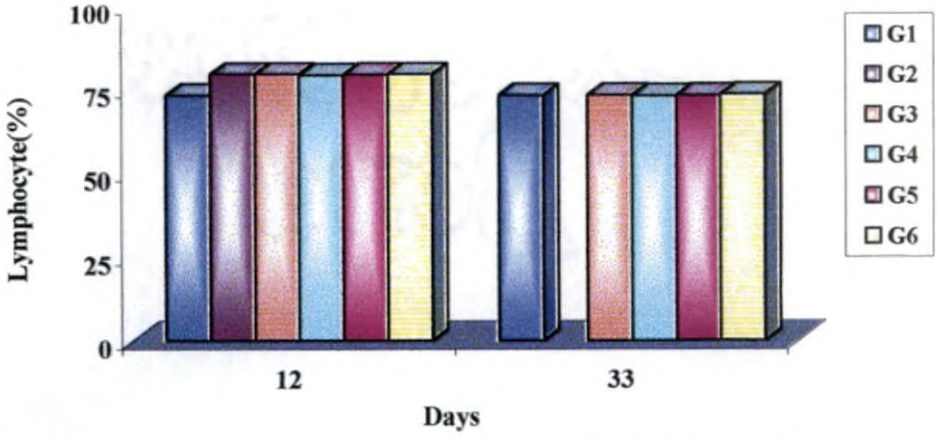


Fig.19. Lymphocyte count of experimental groups maintained on different treatments

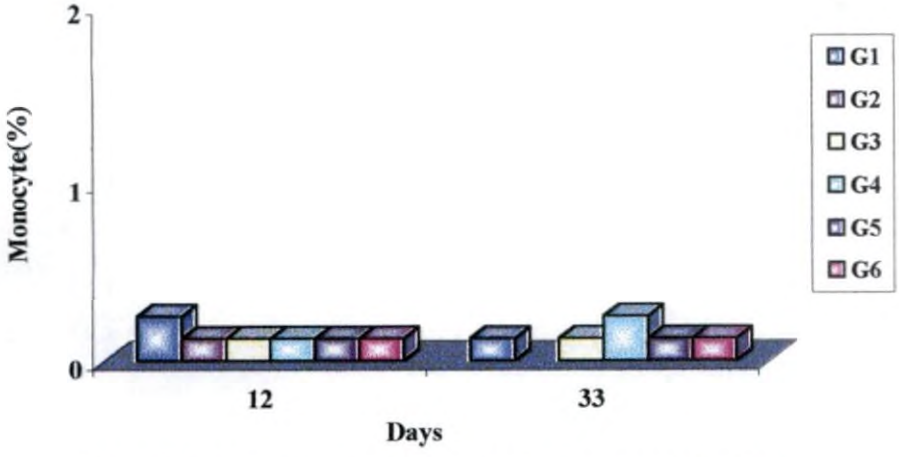


Fig. 20. Monocyte count of experimental groups maintained on different treatments

Discussion

5. DISCUSSION

The objectives of the study included the evaluation of nephroprotective effect of *Aerva lanata* (Cherula) and *Boerrhavia diffusa* (Thazhuthama) against the gentamicin-induced nephrotoxicity in rats.

5.1 FEED INTAKE

In the saline control (G1), the mean feed intake ranges from 104 to 130 g / day. There was no marked variation in the feed intake within the group at different period of time interval. In all other experimental groups (G2, G3, G4, G5 and G6) the feed intake was reduced to 20 g at 12^{th} day. This may be due to, loss of appetite, stress, pain and ureamia produced by gentamicin nephrotoxicity.

Dantas *et al.* (1997) investigated that dogs received 10 mg gentamicin/kg intramuscularly three times a day for 14 days showed loss of appetite, apathy, polyuria, polydipsia, diarrhoea, vomition and oliguria.

An increase in feed intake was observed from 13^{th} day onwards after starting oral administration of *Aerva lanata* @ 250 and 500 mg/kg (G3 and G4) and *Boerrhavia diffusa* @ 250 and 500 mg/kg (G5 and G6). Both *Aerva lanata* and *Boerrhavia diffusa* at two different doses (250 and 500 mg/kg) reverted the feed intake to normal only after two weeks indicate that, there is no wide variation in response between these two different doses.

5.2 BODY WEIGHT

There was no significant difference in the mean body weight of saline control (G1) between and within the group at different period of time interval. A marked decrease in the body weight was observed at 12^{th} day in all other

experimental groups (G2, G3, G4, G5 and G6). This decrease in body weight may be due to decrease in feed consumption.

From the third week of study (19th day), an increase in the body weight was noticed with all the treatment groups (G3, G4, G5 and G6).

Vetrichelvan and Jegadeesan (2002) reported that the ability of aqueous extract of *Aerva lanata* (500 mg/kg body weight) to protect the body weight loss seems to be due to its anti-diabetic activity.

The mean body weight of *Aerva lanata* @ 250 mg/kg (G3) was 211.25 ± 5.15 (0 day), reduced to 171.25 ± 9.15 (12th day). Even though it increased to 187.5 ± 9.77(33rd day) could not gain the original body weight as on the 0 day. But the body weight was reverted to normal in the *Aerva lanata* @ 500 mg/kg body weight (G4). This shows that *Aerva lanata* @ 500 mg/kg is having better efficacy in body weight gain than *Aerva lanata* @ 250 mg/kg body weight.

In case of *Boerrhavia diffusa* (250 and 500 mg/kg body weight), it is having significant increase in body weight gain, but the gain in body weight was not reverted to normal body weight (0 day). Further there was no significant change in increasing the body weight gain between the doses 250 and 500 mg/kg, shows that these two doses are not having marked variation in influencing body weight gain.

5.3 BLOOD UREA NITROGEN

The estimation of blood urea nitrogen level is very important, since it helps in assessing the normal function of the kidney.

There was no variation in the mean blood urea nitrogen level of saline control (G1) between and within the group at different period of time interval.

The mean blood urea nitrogen level of G2, G3, G4, G5 and G6 (0 day) were 27.33 ± 2.05, 28.51 ± 0.32, 27.91 ± 0.22, 26.96 ± 0.20 and 27.71 ± 0.17 respectively. It was elevated to 287.31 ± 9.58, 282.71 ± 2.64, 284.19 ± 2.37, 292.42 ± 4.33 and 292.07 ± 3.90 respectively.

Gupta and Verma (1998) reported that blood urea nitrogen concentrations increased in guinea pigs following intramuscular administration of gentamicin at the dose rate of 4 mg/kg body weight for seven days.

Naidu *et al.* (2000) found blood urea nitrogen and serum creatinine were increased by 896 and 461 per cent respectively with gentamicin compared with the control.

This elevated blood urea nitrogen is due to the gentamicin nephrotoxicity. Hence it is confirmed that gentamicin at a dose rate of 80 mg/kg body weight produces significant nephrotoxicity as evidenced by increase in blood urea nitrogen level.

Bajpai *et al.* (1999) evaluated that administration of graded doses of gentamicin to adult wistar strain rats at doses of 20, 40 and 80 mg/kg/day intramuscularly for seven days induced nephrotoxicity, exhibited by elevated plasma creatinine and blood urea nitrogen concentration.

Vijaykumar *et al.* (2000) confirmed that gentamicin at a dose rate of 80 mg/kg/day subcutaneously for 12 days in rats produced significant nephrotoxicity as evidenced by increase in blood urea nitrogen, serum creatinine and decreased creatinine clearance.

The elevated blood urea nitrogen was started reducing from the subsequent week onwards (19^{th} day). It was about 42.57 ± 1.16, 35.24 ± 0.66 and

67.37 ± 1.81, 48.87 ± 0.72 at 250 and 500 mg of *Aerva lanata* and *Boerrhavia diffusa* respectively.

Fry *et al.* (1977) stated that gentamicin at a normal clinical dose produced greater and more rapid elevations of the blood urea nitrogen in guinea pigs although all elevations were reversible.

Shamim *et al.* (1999) reported that *Bonadequl Buzoor*, a celebrated Unani formulations decreased the serum urea and creatinine level in rats significantly, which were increased by gentamicin administered intramuscularly at the dose rate of 40 mg/kg body weight twice a day for seven days.

Changes in blood urea, serum creatinine and creatinine clearance induced by gentamicin were significantly prevented by the extract (300 mg/kg P.O.). Thus they concluded, *Ginkgo biloba* extract protected rats from gentamicin induced nephrotoxicity (Naidu *et al.*, 2000).

Ali and Mousa (2001) observed that dimethyl sulfoxide (DMSO) dose dependently lowered the elevated plasma urea level, creatinine concentrations and the rise in cortical thiobarbituric acid reactive substances (TBARS) and suggested that dimethyl sulfoxide (25%) has potential protective effects against gentamicin nephrotoxicity in rats.

Blood urea nitrogen level was reverted to normal at 26th day in both *Aerva lanata* @ 500 mg/kg (G4) and *Aerva lanata* @ 250 mg/kg (G3) whereas it was reverted to normal only at 33rd day in *Boerrhavia diffusa* @ 500 mg/kg (G6) and *Boerrhavia diffusa* @ 250 mg/kg (G5). Hence the efficacy of nephroprotective effect of *Aerva lanata* is better than *Boerrhavia diffusa* and doses are not having significant variation in influencing nephroprotective effect.

5.4 MICROSCOPICAL EXAMINATION OF URINE

Presence of hyaline casts, granular casts and urate crystals in urine (12^{th} day) of all experimental groups (G2, G3, G4, G5 and G6) except saline control (G1) indicate the extent of gentamicin nephrotoxicity.

Dogs received 10 mg gentamicin/kg intramuscularly three times a day for 14 days, showed polyuria, polydipsia and oliguria. Laboratory findings included enzymuria, crystalluria, azotaemia and isosthenuria (Dantas *et al.*, 1997).

Only few epithelial cells and hyaline casts were seen at 33^{rd} day, in all the treatment groups (G3, G4, G5 and G6), indicate the regeneration of the renal tubules.

5.5 GROSS AND HISTOPATHOLOGICAL EXAMINATION

The severity of both the gross and microscopic lesions of kidney was observed at 12^{th} day in all the gentamicin treated groups (G2, G3, G4, G5 and G6).

Diffusely distributed greyish white patches in the parenchyma and moderate enlargement of the kidney revealed the extent of damage of kidney at 12^{th} day. These changes are similar to those described by Dantas *et al.* (1997).

Degeneration of cortical tubules, glomerular necrosis, medullary congestion along with focal accumulation of inflammatory cells reflects the gentamicin nephrotoxicity. These changes may be due to the accumulation of gentamicin in this epithelium.

El-Mouedden *et al.* (2000) reported that the apoptosis in the proximal tubules of kidney cortex after gentamicin administration is probably related to its capacity to concentrate in this epithelium.

Choi *et al.* (2000) concluded that long term exposure to gentamicin in the renal proximal tubule-like cell line, LLC-PK, induces apoptosis of the renal tubular epithelial cells and this process may contribute to some of the aminoglycosides nephrotoxicity.

Gentamicin at a dose rate of 80 mg/kg/day subcutaneously for 12 days produced significant nephrotoxicity as evidenced by renal tubular necrosis (Vijaykumar *et al.*, 2000).

Gentamicin at a dose rate of 80 mg/kg/day subcutaneously for 12 days produced significant nephrotoxicity as evidenced by decrease in urea clearance and tubular epithelial damage with intense granular degeneration, more fatty changes and severe necrosis (Samiulla and Harish, 2001).

The exact mechanism by which gentamicin induces the renal damage is unknown, however, evidence suggests a role of reactive oxygen species in this damage, it has been found that O_2, H_2O_2 and hydroxyl radicals increase with gentamicin treatment (Cuzzocrea *et al.*, 2002).

Diffuse tubular degeneration, occasional areas of coagulation of parenchyma and tubular haemorrhage (33rd day) showed that kidney was not recovered completely. But presence of mononuclear cell infiltration and tubules bound by more than one layer of cells indicate that regeneration of renal epithelium has started. This may be due to the presence of active principle aervitrin and amyrin in *Aerva lanata* and Punarnavine in *Boerrhavia diffusa.* Complete regeneration of renal epithelium may be possible, if the extract was administered for longer duration.

Chopra *et al.* (1958) reported that the active principle of *Boerhaavia diffusa* was a compound of alkaloidal nature called 'punarnavine'. Large

quantities of potassium nitrate and other salts contained in the plant might contribute to its diuretic effect.

These principles are having high diuretic activity, which may favour the excretion of gentamicin, results in reduced accumulation of gentamicin in the renal epithelium, thereby reducing gentamicin nephrotoxicity.

Trease and Evans (1985) stated that flavanoids have been shown to possess diuretic activity in rats.

Udupihillie and Jiffry (1986) stated that extract of the whole herb, *Aerva lanata* produced a significant diuresis when compared to water and to isotonic saline and the leaves of the herb produced a much more intense diuresis than that produced by the whole plant.

The alcoholic extract of *Aerva lanata* (800 mg/kg) in rats, increased the urine volume moderately, acted as a potent kaliuretic in dose dependent manner, justified the usefulness of this plant in the treatment of renal dropsies (Vetrichelvan *et al.*, 2000).

Nadkarni (1976) opined that 1-4 drachms of the liquid extract from the plant *Boerhaavia diffusa* produced diuresis in cases of oedema and ascites especially due to early liver, peritoneal and kidney conditions. The diuresis was said to be mainly due to the action of the alkaloid punarnavine.

Srivastava *et al.* (1998) described various chemical constituents of *Boerhaavia diffusa* and reported that some (punarnavine) of them possessed hepatoprotective, diuretic and antiviral properties.

Thus *Aerva lanata* @ 250 and 500 mg/kg (G3 and G4) and *Boerrhavia diffusa* @ 250 and 500 mg/kg (G5 and G6) is having some nephroprotective action on renal epithelium of rats with gentamicin induced nephrotoxicity. But the exact mechanism of action is yet to clear.

As a protective mechanism the cell isolates and subsequently ejects gentamicin injured or possibly gentamicin bound cytoplasmic structures in the form of myeloid bodies (Fowler *et al.*, 1974).

Meister (1981) reported that glutathione peroxidase (GSH) was responsible for protection of cells against oxidative stresses.

Administration of DL alpha-lipoic acid (25 mg/kg/day) orally prevents lipid peroxidation which may atleast partly, play an important role in the injury cascade of gentamicin-induced nephrotoxicity in rats (Sandhya *et al.*, 1995).

Naim *et al.* (1999) reported that due to their anti-oxidant activity vitamin-E (250 mg/kg i.m) and probucol (60 mg/i.m) have potential protective effects in rats against gentamicin nephrotoxicity.

Chaverri *et al.* (2000) stated that reactive oxygen species were involved in gentamicin nephrotoxicity. The protective effect of garlic (2% garlic diet) in rats was associated with the prevention of the decrease of glutathione peroxidase activities and with the rise of lipoperoxidation in renal cortex.

Fifty per cent ethanolic extract of the whole plant of *Solanum nigrum* exhibited significant hydroxyl radical scavenging potential, thus suggesting its probable mechanism of cytoprotection against gentamicin induced kidney cell (vero cells) damage (Kumar *et al.*, 2001).

Simultaneously, a preliminary study was conducted to assess the natural cure of gentamicin nephrotoxicity and also the effect of gentamicin along with the administration of plant extract. The results showed a poor natural recovery, but *Aerva lanata* @ 500 mg/kg plus gentamicin (80mg/kg/day subcutaneously) administered group is having prominent nephroprotective effect which necessitates a detailed study in this aspect.

5.6 HAEMATOLOGY

Gupta and Verma (1998) reported that leukocyte count was decreased and blood urea nitrogen concentrations increased in guinea pigs following intramuscular administration of gentamicin at the dose rate of 4 mg/kg body weight for seven days. No significant effect on packed cell volume, total erythrocyte count and haemoglobin concentration were observed.

The packed cell volume, haemoglobin concentration, total erythrocyte count, eosinophil count and monocyte count of experimental groups showed no significant difference between the group at different period of time interval. All the values fell within the normal range (Hrapkiewicz *et al.*, 1998).

There was significant decrease in the total leukocyte, neutrophil count but a marked increase in lymphocyte count was observed in all the gentamicin administered groups at 12^{th} day (G2, G3, G4, G5 and G6) as compared to saline control (G1). This shows that gentamicin is also having effect on haemopoietic system.

The leukopenia observed in gentamicin administered guinea-pigs might be either due to direct cytotoxic effect of the antibiotic or by acting the antibiotic as an antigen in combination with leukocytic protein thereby resulting in lysis or agglutination of the leukocytes (Benjamin, 1978).

Fadel and Larkin (1996) reported that the leukopenia might be due to necrosis and inflammation at injection sites of gentamicin administration.

The haemogram was reverted to normal at 33rd day in all the experimental groups (G3, G4, G5 and G6). This shows that both *Aerva lanata* and *Boerrhavia diffusa* is having some influence in the haemopoietic system.

Geetha and Sangeetha (2000) conducted a controlled experimental study to assess the effect of *Boerhaavia diffusa* extract at the dose rate of 2.4 g/kg body weight orally to ward off post surgical infection and mortality in albino rats. The results showed that the drug caused maintenance of total and differential leukocyte count after surgery and infection.

Based on the results of experimentation, it can be concluded that alcoholic extract of *Aerva lanata* and *Boerrhavia diffusa* possess nephroprotective effect, where *Aerva lanata* is having better effect than *Boerrhavia diffusa.* Among the doses, *Aerva lanata* @ 500 mg/kg is having prominent nephroprotective effect than other doses.

Therefore the present observations stress on the need for further studies to evaluate the mechanism of action of nephroprotective effect. Moreover, the studies should be directed to evaluate the curative effect by administering the plant extract for longer period rather than evaluating the prophylactic activity, which was done in most of the studies under these plants. Also studies on toxic effects of these plants on various systems/organs should be carried, when administered orally for longer duration.

Summary

6. SUMMARY

A study was conducted to validate the folklore use of *Aerva lanata* (Cherula) and *Boerrhavia diffusa* (Thazhuthama) as nephroprotective agents against gentamicin induced nephrotoxicity in rats.

The experiment was conducted in 48 adult albino rats weighing 150-250 g of either sex. The rats were divided into six groups and each groups consisting of eight rats.

All the experimental animals except saline control (G1) were given gentamicin sulphate at the dose rate of 80 mg/kg/day subcutaneously for 12 days continuously and produced nephrotoxicity, whereas saline control (G1) was given normal saline only.

After 12 days, the experimental groups (G3, G4, G5 and G6) were given two selected doses (G3 and G5 – 250 mg/kg) and (G4 and G6 – 500 mg/kg) of dried alcoholic extract of the two plant *Aerva lanata* and *Boerrhavia diffusa* orally, for a period of another 21 days respectively.

The main items of observations included assessment of feed intake, body weight, blood urea nitrogen, microscopical examination of urine, gross and histopathological examination of kidney and haematological examination.

The feed intake and body weight was gradually reduced in all gentamicin-administered groups (G2, G3, G4, G5 and G6) upto 12^{th} day and it reverted to normal in all the treatment groups (G3, G4, G5 and G6) at 33^{rd} day.

Blood urea nitrogen level was elevated in all gentamicin administered groups (G2, G3, G4, G5 and G6) at 12^{th} day and normalization of blood urea

nitrogen level was observed at 26th day in case of Aerva lanata @ 250 and 500 mg/kg (G3 and G4) and at 33rd day in *Boerrhavia diffusa* @ 250 and 500 mg/kg (G5 and G6).

Presence of only few epithelial cells and hyaline casts in microscopical examination of urine in all treatment groups at 33rd day indicates the initiation of regeneration of renal epithelium.

Gross examination of kidney revealed, circumscribed greyish white patches in the parenchyma and moderate enlargement of kidney, whereas histopathological lesions showed degeneration of the cortical tubules, hyalinization and occasional cystic dilatation of tubules, glomerular necrosis, medullary congestion along with focal accumulation of inflammatory cells in gentamicin sulphate control (G2) confirmed the nephrotoxicity of gentamicin at the dose rate of 80 mg/kg/day i.m.

Presence of mononuclear cell infiltration and tubules bound by more than one layer of cells confirmed that, initiation of regeneration of renal epithelium has started at 33rd day.

There was no marked variation in packed cell volume, haemoglobin concentration, total erythrocyte count, eosinophil count and monocyte count of the experimental groups at 12th and 33rd day of experiment.

Total leukocyte count and neutrophil count was decreased significantly, whereas lymphocyte count was increased at 12th day and haemogram reverted to normal in all the groups at 33rd day.

From the experiment, it was concluded that both *Aerva lanata* (Cherula) and *Boerrhavia diffusa* (Thazhuthama) is having nephroprotective action and hence it could be used as nephroprotective agents.

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NEPHROPROTECTIVE EFFECT OF *Aerva lanata* (Cherula) AND *Boerrhavia diffusa* (Thazhuthama) IN RATS

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ABSTRACT

An experiment was conducted to assess the nephroprotective effect of *Aerva lanata* (Cherula) and *Boerrhavia diffusa* (Thazhuthama) against gentamicin induced nephrotoxicity in rats. A group of 48 adult albino rats weighing 150-250 g of either sex were include in six groups and each group consisting of eight rats.

All the experimental groups except saline control (G1) were given gentamicin sulphate (80 mg/kg/day subcutaneously) for 12 days. After 12 days, the experimental groups (G3, G4, G5 and G6) were given two selected doses [(G3 and G5 – 250 mg/kg) and (G4 and G6 – 500 mg/kg)] of dried alcoholic extract of *Aerva lanata* and *Boerrhavia diffusa* orally, for a period of another 21 days respectively.

Feed intake and body weight was reduced in gentamicin administered groups (G2, G3, G4, G5 and G6) at 12th day and it was reverted to normal at 33rd day (G3, G4, G5 and G6).

Elevated blood urea nitrogen (12th day) was reverted to normal at 26th day in *Aerva lanata* @ 250 and 500 mg/kg (G3 and G4) and at 33rd day in *Boerrhavia diffusa* @ 250 and 500 mg/kg (G5 and G6) respectively.

Microscopical examination of urine at 33rd day, revealed the initiation of regeneration of renal epithelium.

The severity of gross and microscopical lesions of kidney observed at 12th day was minimized at 33rd day, indicate the nephroprotective effect of *Aerva lanata* and *Boerrhavia diffusa*.

The change in haemogram at 12^{th} day (reduced total leukocyte count, neutrophil count and increased lymphocyte count) was reverted to normal in all the groups at 33^{rd} day.

Hence the study confirmed that *Aerva lanata* and *Boerrhavia diffusa* posses nephroprotective action and *it* could be used as nephroprotective agents.

Appendix

APPENDIX – I

Preparation of reagents for the estimation of blood urea nitrogen

a. Reagent A

Dissolved five gram of ferric chloride in 20 ml of water. Transferred this to a graduated cylinder and added 100 ml of orthophosphoric acid (85%) slowly with stirring. Made up the volume to 250 ml with water.

b. Reagent B

Added 200 ml of concentrated sulphuric acid to 800ml water in a two-litre flask slowly with stirring keeping it ice bath.

c. Acid reagent

Added 0.5 ml of reagent A to one litre of reagent B.

d. Reagent C

Transferred 20 g of diacetyl monoxime in one litre of water and filtered it.

e. Reagent D

Added thiosemicarbazide five gram per litre of water.

f. Colour reagent

Mixed 67 ml of C and 67 ml of D and made it upto 1000 ml with distilled water.

g. Stock urea standard

Added 100 mg of urea per 100 ml distilled water.

h. Working standard urea

Diluted 1 ml stock to 100 ml distilled water.

All the reagents were kept in brown bottles at 4°C till use.