# NEPHROPROTECTIVE EFFECT OF *Hygrophila spinosa* AND *Mangifera indica* IN ALBINO RATS

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

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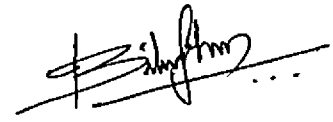
## DECLARATION

I hereby declare that the thesis entitled **"Nephroprotective effect of *Hygrophila spinosa* and *Mangifera indica* in albino 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 **"Nephroprotective effect of *Hygrophila spinosa* and *Mangifera indica* in albino rats"** is a record of research work done independently by **Dr. Bibu John Kariyil,** 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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*BIBU JOHN KARIYIL*

*Dedicated to God*

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# *Introduction*

# 1. INTRODUCTION

Nephrotoxicity has been caused by several xenobiotic substances damaging renal proximal tubule, the portion of the nephron with greater sensitivity to nephrotoxic effects. Some of the chemicals which cause damage to the proximal tubule are antibacterial agents such as cephaloridine and aminoglycosides, anticancer drugs such as cisplatin and industrial chemicals such as cadmium, hexavalent chromium, mercury and palladium (Cristofori *et al.*, 2007). 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 defective drug excretion adversely affects the different systems of the body. Since kidneys are the major route of drug excretion, the occurrence of nephrotoxicity is of great concern.

Nephrotoxicosis has been reported in 15 per cent of human patients treated with gentamicin. In Veterinary medicine the frequency of this toxicity has not been documented except in dogs. Horses are highly sensitive to nephrotoxicosis as are cats (Lashev and Lasarova, 2001).

Nephrotoxicity is caused by the accumulation of aminoglycosides in the renal proximal tubular cells and these get localized within the endosomal and lysosomal vacuoles (Mingeot-Leclercq and Tulkens, 1999). Recently, it is proposed that aminoglycoside nephrotoxicity results from endocytic retrieval of the drugs by megalin, a 600-kDa endocytosis – mediated receptor, with subsequent sequestration in the lysosomes of proximal tubular cells, leading ultimately to lysosome rupture and necrotic cell death (Ward *et al.*, 2005).

Generation of reactive oxygen metabolites has been one of the major contributing factors towards nephrotoxicity (Baliga *et al.*, 1997, Walker *et al.*, 1999). Some antioxidants and free radical scavengers such as polyaspartic acid (Ramsammy

*et al.*, 1989), probucol (Vijaykumar *et al.*, 2000), melatonin (Shifow *et al.*, 2000), dimethyl sulfoxide (Ali and Mousa, 2001) caffeic acid phen ethyl ester (Vardi *et al.*, 2005) have been reported to attenuate gentamicin-induced renal failure. The growing awareness of the secondary effects of these nephroprotective agents made people explore the time tested remedies from traditional alternative medicine.

'Ayurveda' (Devanagari) is an ancient traditional system of medicine which is native to the Indian subcontinent. It describes many alternatives to the synthetic drugs having side effects. The herbal drugs used in this traditional system are found to be effective with minimal or no side effects.

There has been a tremendous boom in the field of herbal medicine development in recent times. Many plants have been assessed for their action in various systems of the body. More than 13,000 plants have been studied during the past five years (Dahanukar *et al.*, 2000). India, being a treasure trove of medicinal plants, contributes much to these. Many regions in Kerala, especially the Western Ghats, still remain to be explored for unidentified medicinal herbs.

In order to make these traditional remedies acceptable to modern physicians, there is a need to identify the active principles of these herbal drugs with the knowledge of their specific mechanism of action, in order to validate their effects scientifically.

In this study, an attempt has been made to evaluate the nephroprotective effect of *Hygrophila spinosa* and *Mangifera indica* against gentamicin-induced nephrotoxicity in albino rats.

*Hygrophila spinosa*, T. Anders. (Kokilaksha in Sanskrit and Vayalchulli in Malayalam) Syn. *Asteracantha longifolia,* Nees., Syn. *Hygrophila auriculata* (K.Schum.) Heine., Syn. *Hygrophila schulli* (Ham.)(Sasidharan and Sivarajan, 1996) is a well known medicinal plant (family: Acanthaceae) found in paddy fields and marshy areas. The whole plant has medicinal properties and it is being used in

ayurveda as a diuretic, aphrodisiac and in the treatment of dropsy, scanty urine and ascites (Nadkarni, 1976).

*Mangifera indica* Linn. belonging to the family Anacardiaceae has many medicinal properties. The fruit is used as a laxative, diuretic, astringent and the bark is used in the treatment of uterine haemorrhagic ailments (Chopra *et al.*, 1956).

The information gathered by this study would help for assessing the nephroprotective effect of these plants thereby recommending them for the treatment of various nephrotoxicosis in animals and man.

# *Review of Literature*

# 2. REVIEW OF LITERATURE

## 2.1. NEPHROTOXICITY

Frame *et al.* (1973) reported that rabbits receiving gentamicin had a greater degree of kidney damage if the drug was given in divided doses rather than a single daily injection.

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 observed on histopathological examination (Welles *et al.*, 1973).

Luft *et al.* (1976) concluded that gentamicin at a dose of 5 mg/kg subcutaneously at 4 hour interval in rats produced polyuria, decrease in creatinine clearance, decline in urine osmolality, proteinuria and an increase in the excretion of lysosomal enzymes.

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.

Hottendorf and Gordon (1980) observed that gentamicin at a dose of 20 mg/kg subcutaneously, twice a day, for 28 days in rats produced nephrotoxicity with moderate degree of bilateral proximal tubular necrosis.

Gentamicin showed a greater accumulation tendency in the renal parenchyma even in sub toxic doses (10 mg/kg twice daily subcutaneously) in rats indicating its greater affinity for proximal tubular cells. The data also demonstrated that gentamicin given frequently accumulated more rapidly and to higher renal levels than did the same dose given at a longer dose interval (Aronoff *et al.*, 1983).

Deb *et al.* (1986) compared the nephrotoxic effect of ampicillin and tetracycline in rats. Ampicillin at a dose of 30 mg/kg intramuscularly for 7 days increased the serum and urinary alkaline phosphatase, acid phosphatase, creatinine and urea indicating the nephrotoxic action of the antibiotic. The study concluded that ampicillin was less nephrotoxic than tetracycline.

Beauchamp *et al.* (1997) opined that gentamicin at a dose of 40 mg/kg/12h subcutaneously for 10 days in rats induced a dose- and time- dependent renal toxicity as evaluated by increased accumulation of gentamicin in the renal cortex, decreased sphinomyelinase activity, histopathologic and morphometric analysis (proximal tubular necrosis), elevated blood urea nitrogen and serum creatinine levels and higher cellular regeneration ([$^3$H] thymidine incorporation into DNA of cortical cells).

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 a 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 was observed. Histopathology revealed mild nephrotoxic lesions in kidneys after gentamicin administration (Gupta and Verma, 1998).

Vijaykumar *et al.* (2000) confirmed that intraperitoneal injections of gentamicin at a dose of 80 mg/kg for eight days in rats produced significant nephrotoxicity as evidenced by increase in blood urea and serum creatinine, decreased creatinine clearance and renal tubular necrosis. Gentamicin treated rats showed tubular epithelial damage with intense granular degeneration involving more than 50 per cent of renal cortex.

The study conducted by Ali *et al.* (2001) indicated that male Sprague-Dawley rats were more vulnerable to gentamicin nephrotoxicity (80 mg/kg intraperitoneally for six days) than the female ones of the same strain.

Ali *et al.* (2004) revealed that gentamicin nephrotoxicity induced at a dose of 80 mg/kg intramuscularly for six days in rats was potentiated by Spironolactone treatment.

The effect of non selective and selective cyclooxygenase inhibitors on gentamicin induced nephrotoxicity (100 mg/kg intraperitoneally for 5 days) was studied in rats by Hosaka *et al.* (2004). The results revealed that non selective cyclooxygenase inhibitor, indomethacin, produced more intense renal damage when compared with selective cyclooxygenase inhibitor, rofecoxib, suggesting a potentiation of nephotoxicity of indomethacin with gentamicin.

Even though acidic phospholipids in brush border membrane of kidney may serve as the initial binding site for aminoglycosides, Nagai and Takano (2004) opined that Megalin, a giant endocytic receptor, member of low density lipoprotein receptor gene family, expressed abundantly at the apical membrane of the renal proximal tubules, played an important role in binding and endocytosis of amino glycosides into the proximal tubular cells and thereby inducing nephrotoxicity.

Ghaznavi *et al.* (2005) studied the effects of inhibition or induction of nitric oxide synthase on gentamicin-induced acute renal failure in isolated perfused rat kidneys. Gentamicin at a dose rate of 0.5 mg/ml of perfusate produced nephrotoxicity as evidenced by increase in urinary N-acetyl-β-D-glucosaminidase, lactate dehydrogenase and alkaline phosphatase enzyme activities. L-arginine infusion (2 mM in perfusate) in intact isolated rat kidneys prevented gentamicin-induced renal damage by inducing nitric oxide synthase.

Cristofori *et al.* (2007) measured the entity of damage caused by renal proximal tubule segment-specific nephrotoxicants such as potassium dichromate

($K_2Cr_2O_7$), hexachloro-1,3-butadiene (HCBD) and cisplatin in male Wistar rats. The results proved that 24 and 48 hours after treatment, chemicals that selectively affected the $S_3$ segment of the proximal tubule namely HCBD and cisplatin, caused diffused necrosis of the segment with a corresponding decrease in glutamine synthetase activity in the kidney cortex. Potassium dichromate exclusively damaged $S_1$-$S_2$ segments causing diffuse necrosis. No impairment of p-aminohippuric acid (PAH) uptake was observed, even though slight but significant decrease was caused by HCBD 48 hours after treatment.

Kaynar *et al.* (2007) examined the protective effect of N–acetyl cysteine against amikacin mediated nephropathy in Balb/c type mice. N–acetyl cysteine administration reduced granulovacuolar tubular degeneration, myeloid body formation, mitochondrial electron–dense material deposition and mitochondrial swelling in tubule epithelium which occurred as a result of amikacin treatment.

Kozat *et al.* (2007) investigated the effect of gentamicin-induced nephrotoxicity on various biochemical parameters in male Sprague-Dawley rats. Gentamicin at 80mg/kg intraperitoneally for 8 days produced significant nephrotoxicity as evident by increase in serum urea (60.2 ± 5.1 mg/dl) and serum creatinine (2.33 ± 0.05 mg/dl) levels with respect to control values which were 34.5 ± 0.4 mg/dl, 0.24 ± 0.01 mg/dl respectively. The lipid peroxidation, kidney glutathione, glutathione peroxidase, superoxide dismutase levels were 1.05 ± 0.05 nmol/ml, 24.21 ± 1.5 mg/dl, 55.2 ± 4.4 mU/ml, 4.32 ± 0.27 U/ml respectively when compared with normal isotonic saline treated group which were 0.72 ± 0.025 nmol/ml, 39.89 ± 24 mg/dl, 120.4 ± 7.4 mU/ml, 8.10 ± 0.26 U/ml respectively.

## 2.2. NEPHROPROTECTIVE AGENTS

Humes *et al.* (1984) proved that calcium ions appeared to be an effective competitive inhibitor of gentamicin-renal membrane binding interactions and oral calcium loading (4 per cent calcium carbonate in diet) significantly protected against

later stages of gentamicin nephrotoxicity in male Sprague-Dawley rats by the inhibitory effect of calcium ions at subcellular membrane sites of renal proximal tubules. In their experiment, the serum calcium levels in standard diet and calcium supplemented diet groups were 2.97 ± 0.45 mM and 2.89 ± 0.17 mM respectively.

Ramsammy *et al.* (1989) confirmed that polyaspartic acid protected rat kidney against gentamicin-induced proximal tubular necrosis without inhibiting the renal accumulation of the drug. Polyaspartic acid decreased serum creatinine and increased creatinine clearance, decreased N-acetyl-β-D-glucosaminidase excretion in urine, provided a slight increase in catalase activity and reduction in thiobarbituric acid reactive substances in the renal cortex.

Abdel-Naim *et al.* (1999) evaluated that pre treatment with Vitamin E (250 mg/kg intramuscularly) and probucol (60mg/kg intramuscularly) significantly lowered the serum urea and creatinine levels, urinary activity of N-acetyl-β-D-glucosaminase and γ-glutamyl-transferase, ameliorated the rise in renal content of malondialdehyde and enhanced the renal non protein sulphydryl content as well as superoxide dismutase activity against gentamicin nephrotoxicosis in rats. Simultaneous use of Vitamin E and probucol proved to be more effective in mitigating the disturbances in the assessed parameters.

Quercetin, a plant bioflavonoid was assessed for its cytoprotective activity in cisplatin-induced cell injury in rat kidney. Cisplatin was found to increase lipid peroxidation and decrease the activities of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase. Concomitant dose of cisplatin (3mg/kg intraperitoneally) and quercetin (20 mg/kg intraperitoneally) was found to reverse these changes (Devipriya and Shyamala Devi, 1999).

Shifow *et al.* (2000) observed that administration of melatonin (5mg/kg intra gastrically for 11 days) provided protection in rats against gentamicin-induced

nephrotoxicity by inhibiting lipid peroxidation, restoring the antioxidant levels (superoxide dismutase, catalase and glutathione peroxidase) and also by regulating calcium channels. Melatonin also reduced blood urea (23 ± 2.7 mg/dl) and serum creatinine (0.88 ± 0.19 mg/dl) levels when compared with gentamicin controls (289 ± 50 mg/dl and 2.5 ± 0.5 mg/dl respectively).

Ali and Mousa (2001) found that dimethyl sulfoxide, an aprotic solvent, 50 per cent level v/v in normal saline, protected rat kidney against gentamicin-induced nephrotoxicity (100 mg/kg intramuscularly for 6 days). The nephroprotection was attributed to the scavenging activity of the free radicals that were generated from gentamicin toxicity.

Pedraza-Chaverri *et al.* (2003) pointed that diallyl sulfide, a garlic derived compound with antioxidant properties, at a dose of 50 mg/kg/day for 4 days intra gastrically provided protective effect on oxidative stress and gentamicin nephrotoxicosis. Gentamicin increased renal oxidative stress markers nitrotyrosine and protein carbonyl levels in rats which were ameliorated by diallyl sulfide.

Karatas *et al.* (2004) observed that gentamicin led to acute tubular necrosis in the rat kidney and administration of tempol (3.5, 7, 14 mg/kg intraperitoneally), a superoxide dismutase mimetic membrane permeable radical scavenger, protected kidney against gentamicin-induced toxicity in a dose-dependent manner.

Caffeic acid phenethyl ester, an active component of propolis extract at a dose of 10μmol/kg intraperitoneally was found to protect kidney from gentamicin nephrotoxicosis in rats. Caffeic acid phenethyl ester, through its proven antioxidant property, brought out this nephroprotective action by preventing elevation of malondialdehyde levels and histological renal changes induced by gentamicin (Vardi *et al.*, 2005).

Dhanarajan *et al.* (2006) reported the protective effect of Ebselen (1-chloro-2,4-dinitrobenzene), a selenoorganic drug with anti-inflammatory, anti

atherosclerotic, cytoprotective properties which prevented gentamicin-induced renal damage in male Wistar rats. Pre treatment with Ebselen at a dose of 5 mg/kg body weight prevented gentamicin-induced nephrotoxicity. The protective role of Ebselen was attributed to its antioxidant property mimicking glutathione peroxidase and inhibition of gentamicin-induced nitrosative stress.

Silan *et al.* (2007) demonstrated that resveratrol (10 mg/kg intra peritoneally), a natural polyphenol phytolexin, had a protective effect against gentamicin nephrotoxicity in rats. The protective effect of resveratrol was evidenced by its free radical scavenging properties and reduction in lipid peroxidation.

## 2.3. NEPHROPROTECTIVE PLANTS

The references on nephroprotective effect of *Hygrophila spinosa* and *Mangifera indica* are scanty.

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

Cystone, a polyherbal ayurvedic preparation, was investigated for its effect on nephrotoxicity and antitumor activity of cisplatin. The results suggested that pre treatment with cystone (1000 mg/kg intraperitoneally) protected against cisplatin-induced nephrotoxicity without interfering with its anti tumor activity in C57BL/6J mice (Rao *et al.*, 1999).

Pedrazo-Chaverri *et al.* (2000) stated that reactive oxygen species were involved in gentamicin toxicity. The protective effect of garlic (2 per cent garlic diet) in rats was associated with the prevention of the decrease of superoxide dismutase, glutathione peroxide activities and the rise of lipid peroxidation in renal cortex.

Tahri *et al.* (2000) concluded that an acute hypotensive action of aqueous extract of aerial parts of *Urtica dioica* indicated a direct effect on the cardiovascular

system. Moreover, a diuretic and natriuretic effect was also observed in rats at a dose of 24 mg/kg body weight suggesting its action on the renal function.

Venkatesan *et al.* (2000) found that curcumin prevented adriamycin nephrotoxicity in rats. Curcumin (200 mg/kg body weight in 1 per cent gum acacia) inhibited urinary excretion of N-acetyl-β-glucosaminase, fibronectin and glycosaminoglycan. Curcumin restored renal function by increased glomerular filtration rate and suppressed oxidative stress.

Kumar *et al.* (2001) tested *in vitro* the cytoprotective activity of 50 per cent ethanolic extract of the whole plant of *Solanum nigrum* (10, 100, 1000μg/ml) against gentamicin-induced toxicity in Vero cells. The test extract significantly exhibited hydroxyl radical scavenging potential in a dose-dependent manner.

Ali (2002) stated that generation of oxygen free radicals in kidney cortex played an important role in the pathogenesis of gentamicin nephrotoxicity in rats. In his experiment, aqueous extract of *Rhazya stricta* leaves at a dose of 0.5 and 1 mg/kg/day orally produced dose-related amelioration in the indices of gentamicin-induced nephroptoxicity. He concluded that a dose-dependent increase in the cortical concentration of glutathione and superoxide dismutase and a decrease in thiobarbituric acid reactive substances (TBARS) could be the basis for the nephroprotective action.

Arabic gum at the dose rate of 7.5 g/100 ml in drinking water 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 (Al-Majed *et al.*, 2002).

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

Afzal *et al.* (2004) pointed out that increased serum levels of urea and creatinine following gentamicin administration at a dose rate of 40 mg/kg twice daily for 7 days could be significantly reduced by aqueous and ethanolic extracts of *Jawarish Zarooni Sada* (300 mg/kg intragastrically), a known nephroprotective preparation containing 15 herbal ingredients.

Kotnis *et al.* (2004) reported that aqueous slurry of *Hemidesmus indicus* root powder in Wistar rats of both sexes provided nephroprotection indicated by reduction in serum levels of creatinine, gamma glutamyl transpeptidase (γGT) and alkaline phosphatase in gentamicin-induced nephrotoxicity. The histopathology revealed mild to moderate lesions in the proximal tubules and glomerulus of plant-treated group.

The ethanolic extract of *Aerva lanata* (300mg/kg orally) was studied for its nephroprotective activity in gentamicin-induced renal injury in albino rats by Shirwaikar *et al.* (2004). *Aerva lanata* provided nephroprotection by means of flavonoids which were potent antioxidant and free radical scavengers.

The study conducted by Ali *et al.* (2005) revealed that curcumin had a palliative action at a dose of 200 mg/kg/day for 10 days on gentamicin-induced nephrotoxicity. The results suggested that curcumin could ameliorate the histopathological and biochemical indices of nephrotoxicity in rats mainly by its anti oxidant property.

Essa *et al.* (2005) reported that the antihyperammonemic effect of ethanolic extract of leaves of *Pongamia pinnata* (300 mg/kg orally) in rats could be attributed to its nephroprotective effect by means of detoxifying excess urea and creatinine, free radical scavenging and antioxidant properties.

Baek *et al.* (2006) evaluated the cytoprotective principles of *Panax ginseng* using cisplatin-induced nephropathy in renal tubular epithelial cells using activity guided fractionation. Ginsenosides which are the active principles of *Panax ginseng*

increased cell viability and decreased lactate dehydrogenase leakage in a dose-dependent manner, reflecting reduced cisplatin nephrotoxicity in cultured renal proximal tubular epithelial cells.

El-Ashmawy *et al.* (2006) examined the protection conferred by grape seed extract against gentamicin-induced nephrotoxicity and genotoxicity in bone marrow cells of albino rats. They found that pretreatment with grape seed extract at a dose of 150 mg/kg body weight orally for 7 days decreased the serum urea (26.0 ± 2.0), creatinine (0.47 ± 0.01), malondialdehyde content (8 ± 0.3) and increased the activity of glutathione peroxidase (64.5 ± 1.6) and glutathione level (5.0 ± 0.3) when compared with normal.

Investigations were carried out to study the effect of *Spirulina fusiformis*, blue green algae, on gentamicin-induced oxidative stress and renal dysfunction. Kuhad *et al.* (2006) stated that reactive oxygen species generated by gentamicin in rats impaired the expression of endothelial nitric oxide synthase. *Spirulina fusiformis* at doses 500, 1000, 1500 mg/kg, p.o. significantly and dose-dependently restored renal functions (serum creatinine, blood urea nitrogen, creatinine clearance and serum nitrate levels), reduced lipid peroxidation and enhanced reduced glutathione levels, superoxide dismutase and catalase activities.

2.4. *Hygrophila spinosa*

Kumari and Iyer (1967) conducted preliminary studies on the diuretic effects of *Hygrophila spinosa* and *Tribulus terrestris*. According to their observations, 0.3 OsM solutions of aqueous extracts of ashes and decoctions of *Hygrophila spinosa* possessed diuretic activity. The effects were attributed to high potassium content of the plant.

Nadkarni (1976) opined that tincture of whole plant - *Hygrophila spinosa* (1 in 3 of alcohol)given orally in doses of 20 to 30 minims, three times daily was found

beneficial in urinary infections, particularly dysuria and painful micturition. He also reported the diuretic action of root, seed and leaves of the plant.

Mazumdar *et al.* (1997) concluded that petroleum ether extract of *Hygrophila spinosa* at a dose of 60 mg/kg intraperitoneally exhibited antitumor activity in Ehrlich ascites carcinoma and sarcoma-180 bearing mice. The processed extract caused a significant reduction in tumor fluid volume and in the number of living cells.

Mazumdar *et al.* (1999) reported that chemical investigation of petroleum ether extract of *Hygrophila spinosa* root showed the presence of sterol – lupenone, a triterpine alcohol – lupeol, xylose and uronic acids. The pharmacological evaluation of the extract in mice exhibited central nervous system depressant activity which was shown by its anticonvulsant property and potentiation of sedative-hypnotic action at a dose of 80 mg/kg intraperitoneally.

Gomes *et al.* (2001) concluded that the 50 per cent ethanolic extract of aerial parts of *Hygrophila spinosa* at doses 100 and 200 mg/kg, p.o. caused a significant increase in hemoglobin, hematocrit red blood corpuscles and total white blood corpuscles as compared with the saline treated control male albino rats. Serum iron and serum total iron binding capacity were significantly decreased in *Hygrophila spinosa* extract treated rats where these findings demonstrated the heamatinic effect of this plant extract.

Misra *et al.* (2001) reported that *Asteracantha longifolia* contained flavonoids, terpenoids, sterols, betulin, 25-oxo-hentria contanyl acetate and methyl 8-n-hexyltetracosanoate.

Hewawasam *et al.* (2003) stated that the aqueous extract of *Asteracantha longifolia* (0.9 g/kg) provided hepatoprotective action against carbon tetrachloride and paracetamol-induced hepatotoxic models. The protective effect was attributed to

the presence of flavonoids which are known free radical scavengers and antiperoxidants.

According to Shanmugasundaram and Venkataraman (2005), the aqueous extract of aerial parts and roots of *Hygrophila auriculata* exhibited anti-nociceptive activity at 100 and 200 mg/kg p.o. using chemical and thermal methods of nociception in mice. The aqueous extract of aerial parts of *Hygrophila auriculata* exhibited greater writhing inhibition percentage whereas the aqueous extract of root of *Hygrophila auriculata* exhibited greater pain inhibition percentage.

The hepatoprotective activity of the aqueous extracts of roots of *Hygrophila auriculata* was studied on cell induced liver toxicity in Wistar albino mice by Shanmugasundaram and Venkataraman (2006). They stated that a dose of 150 mg/kg p.o. produced significant hepatoprotective action by increasing the activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase and decreasing the total protein content. *In vitro* antioxidant activity was also exhibited by the aqueous root extract.

Vijaykumar *et al.* (2006) reported that the 50 per cent ethanolic extract of aerial parts of *Hygrophila auriculata* possessed antidiabetic and antioxidant properties at doses 100 and 250 mg/kg p.o. in rats.

2.5. *Mangifera indica*

Chopra *et al.* (1956) reported that *Mangifera indica* bark could be used in the treatment of uterine haemorrhage, haemoptysis and malena, diarrheoa and other discharges.

Aderbigbe *et al.* (1999) found out that aqueous extract of *Mangifera indica* (1 g/kg) given orally in rats produced antihyperglycaemic effect in glucose-induced hyperglycaemia but not in streptozotocin-induced hyperglycaemia.

Martinez *et al.* (2000) tested the *in vitro* antioxidant activity of aqueous stem bark extract of *Mangifera indica.* It showed powerful scavenger activity of hydroxyl radicals. The extract also showed a significant inhibitory effect on the peroxidation of rat brain phospholipids and inhibited DNA damage by bleomycin or copper-phenanthrolin systems.

Sanchez *et al.* (2000) found out that decoction of stem bark extract of *Mangifera indica* at a dose of 250 mg/kg administered orally in OF1 mice attenuated 12-o-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative damage in serum, liver and brain as well as prevented the production of reactive oxygen species by peritoneal macrophages.

Garrido *et al.* (2001) established that the dose-dependent analgesic action in CF-1 mice and anti-inflammatory action in rats of the aqueous extract of *Mangifera indica* (20-1000 mg/kg/ p.o.) was due to polyphenols.

Makare *et al.* (2001) established the immunostimulant property of 95 per cent ethanolic extract of stem bark of *Mangifera indica* in swiss albino mice.

QF808, the active ingredient of QF808 formulation, prepared as decoction of stem bark of *Mangifera indica,* contained polyphenols, terpenoids, steroids, fatty acids and micro elements and was proven to be antioxidant due to its ability to scavenge free radicals involved in microsome lipid peroxidation (Martinez *et al.*, 2001).

Prashanth (2001) reported α-glucosidase inhibitory activity (*in vitro*) of the ethanolic extract of *Mangifera indica* bark which might be attributed to the presence of active principles like xanthones, sterols and tannins.

Anila and Vijayalakshmi (2002) reported that flavonoids isolated from the ethyl acetate fraction of dried kernels of *Mangifera indica* effectively reduced lipid levels in serum and liver, heart, kidney and aortic tissues of rats induced hyperlipidemia.

Agbonon *et al.* (2002) noticed that aqueous extract of stem bark of *Mangifera indica* relaxed dose-dependently the rat tracheal smooth muscle strip that was contracted by acetyl choline.

Anila and Vijayalakshmi (2003) observed that oral administration of flavonoids isolated from ethyl acetate fraction of ground dried materials of *Mangifera indica* (10 mg/kg body weight) significantly elevated superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, reduced glutathione and decreased lipid peroxide content in hypercholesterolemic rats.

Investigations carried out by Garcia *et al.* (2003a) revealed the mouse humoral response immunomodulatory activity of aqueous stem bark extract of *Mangifera indica* and Mangiferin. They found that Mangiferin at a dose of 100 mg/kg orally induced an increase in serum antibody levels when compared with the aqueous stem bark extract of *Mangifera indica* which produced an immunosuppressive action at a dose of 500 mg/kg orally.

Garcia *et al.* (2003b) demonstrated that both the aqueous extract of *Mangifera indica* (500 mg/kg orally) and the polyphenol Mangiferin (50 mg/kg orally) had modest anthelminthic and antiallergic activities which was evident by decrease in the number of *Trichinella spiralis* strain typed as GM-1 larvae encysted in the musculature and inhibition of mast cell degranulation.

Sairam *et al.* (2003) revealed that methanolic and aqueous extracts of seeds of *Mangifera indica* 250 mg/kg administered orally possessed anti-diarrhoeal activity in CDI strain mice and part of the activity of the methanolic extract might be attributed to its effect on intestinal transit.

In arachidonic acid and phorbol myristate acetate-induced mouse ear edema models, the decoction of bark extract of *Mangifera indica* administered topically proved to be anti-inflammatory *in vivo* (Garrido *et al.*, 2004).

Garrido *et al.* (2005) proved that decoction of stem bark extract of *Mangifera indica* (25μg/mL) inhibited early and late events in T cell activation *in vitro*, including CD25 cell surface expression, progression to the S-phase of the cell cycle, proliferation in response to T cell receptor stimulation and prevented TNF α-induced IKBα degradation and the binding of nuclear transcription factor, NF-κB to the DNA. This explained the immunomodulatory and anti-inflammatory effects of the extract at the molecular level.

Muruganandan *et al.* (2005) demonstrated the antidiabetic, anti hyperlipidemic and antiatherogenic property of mangiferin, a xanthone glucoside present in *Mangifera indica*, at a dose rate of 10 mg/kg and 20 mg/kg intraperitoneally in streptozotocin-induced diabetic rats.

# *Materials and Methods*

# 3. MATERIALS AND METHODS

## 3.1. EXPERIMENTAL ANIMALS

The study was conducted in 80 adult male Sprague Dawley rats weighing 200 – 250g. 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-h light: 12-h dark cycle. They were maintained under identical feeding and management practices in the laboratory. An acclimatization period of four days was allowed before the commencement of the experiment. The experiment was conducted for a period of 30 days.

## 3.2. PLANT MATERIALS/DRUGS

The whole plant of *Hygrophila spinosa* was collected from the marshy areas of Muringoor, Mukundapuram taluk, Thrissur district, Kerala and identified (Fig.1). The stem bark of locally bred *Mangifera indica* was collected from the campus of College of Veterinary and Animal Sciences, Mannuthy and identified (Fig. 2).

### 3.2.1. Preparation of alcoholic extract of *H. spinosa* and *M. indica*

The whole plant of *Hygrophila spinosa* and stem bark of *Mangifera indica* were air-dried at room temperature and coarsely powdered using an electrical pulverizer. The powders obtained were 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 8.97% and 25.71% respectively on dry matter basis.

Fig. 1. *Hygrophila spinosa* (Vayalchulli)

Fig. 2. Stem bark of *Mangifera indica*

### 3.2.2. Preparation of aqueous extract of *H. spinosa* and *M. indica*

The whole plant of *Hygrophila spinosa* and stem bark of *Mangifera indica* were air-dried at room temperature and coarsely powdered using an electrical pulverizer. 100g of the powder was mixed with one litre of distilled water and kept undisturbed for 24 hours. Then they were subjected to boiling for 30 minutes with constant stirring. The extracts were filtered through a muslin cloth and then kept in boiling water bath for the complete evaporation of the water. The yield of the extract was 14.43% and 30.3% respectively on dry matter basis.

The dried extracts (both ethanolic and aqueous) of *Hygrophila spinosa* and stem bark of *Mangifera indica* were suspended in 0.125% Tween 80 and administered orally to the experimental animals using an orogastric tube.

### 3.2.3. Gentamicin sulphate

Gentamicin sulphate (procured from TTK Pharma Limited, Raja Annamalaipuram, Chennai, India) was administered at a dose rate of 80 mg/kg intraperitoneally for eight days to induce nephrotoxicity.

## 3.3. PHYTOCHEMICAL SCREENING

The ethanolic and the aqueous extracts of whole plant of *Hygrophila spinosa* and stem bark of *Mangifera indica* were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

### *3.3.1. Tests for Detection of Steroids*

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.

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.

Mayer's test

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.

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.

Hager's test

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.

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) 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*

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.

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*

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.

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*

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.

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*

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.

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.

## 3.4. EXPERIMENTAL DESIGN

The animals were randomly divided into 10 groups comprising eight animals each. The experiment was conducted for a period of 30 days. Eight rats were retained as healthy control. Rest of the rats were treated with Gentamicin sulphate at a dose rate of 80 mg/kg intraperitoneally for 8 days.

Group I – Healthy control was administered with vehicle (0.125% Tween 80) p.o. for 30 days.




Group II – Gentamicin sulphate was given at a dose rate of 80 mg/kg intraperitoneally for eight consecutive days.

Group III – Ethanolic extract of *Hygrophila spinosa* was administered at a dose rate of 50 mg/kg p.o. from 9$^{th}$ day to day 30.

Group IV – Ethanolic extract of *Hygrophila spinosa* was administered at a dose rate of 250 mg/kg p.o. from 9$^{th}$ day to day 30.

Group V – Aqueous extract of *Hygrophila spinosa* was administered at a dose rate of 50 mg/kg p.o. from 9$^{th}$ day to day 30.

Group VI – Aqueous extract of *Hygrophila spinosa* was administered at a dose rate of 250 mg/kg p.o. from 9$^{th}$ day to day 30.

Group VII – Ethanolic extract of stem bark of *Mangifera indica* was administered at a dose rate of 100 mg/kg from 9$^{th}$ day to 30$^{th}$ day.

Group VIII – Ethanolic extract of stem bark of *Mangifera indica* was administered at a dose rate of 500 mg/kg p.o. from 9$^{th}$ day to 30$^{th}$ day.

Group IX – Aqueous extract of stem bark of *Mangifera indica* was administered at a dose rate of 100 mg/kg p.o. from 9$^{th}$ day to 30$^{th}$ day.

Group X – Aqueous extract of stem bark of *Mangifera indica* was administered at a dose rate of 500 mg/kg p.o. from 9$^{th}$ day to 30$^{th}$ day.

The blood was collected from all the animals on 0$^{th}$, 9$^{th}$, 15$^{th}$ and 30$^{th}$ day and serum was separated and used for the estimation of creatinine, urea, albumin, total protein, sodium, potassium and calcium.

On 30$^{th}$ day, the rats were sacrificed and both the kidneys were located and dissected out. The kidneys were immediately weighed and the right kidney was used

for the estimation of superoxide dismutase, lipid peroxidation, catalase and the left kidney was used for the estimation of reduced glutathione and 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. Kidney

The animals were euthanized and dissected upon and the kidney 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 Mimami 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. 250 mg of freshly excised kidney was homogenized with 2.5ml 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).

***3.5.1.2. Estimation of Lipid peroxidation***

The levels of lipid peroxidation in kidney tissue were estimated by the method of Fraga (1988).

**a. Reagents**

1. Trichloro acetic acid (TCA) – 15 percent
2. Hydrochoric acid (HCl) - 0.25N
3. Thiobarbituric acid (TBA) – 0.38 percent in hot distilled water
4. TCA-TBA-HCl reagent solution : 1,2 and 3 were mixed freshly in the ratio 1:1:1
5. Stock solution – 4.8mM: 0.079ml of 1, 1', 3, 3' tetra methoxy propane was diluted to 100 ml.

**b. Procedure**

1. 250 mg of kidney tissue was homogenated with Tris-HCl buffer (pH 7.5)
2. 1.0ml of the tissue homogenate was treated with 2.0ml of TBA-TCl-HCl reagent and mixed thoroughly.
3. The mixture was kept in boiling water bath for 15 minutes.
4. After cooling, the tubes were centrifuged at 3200rpm for 10 minutes and the supernatant was taken for measurement.
5. The standard solution was also treated in the similar manner.
6. The absorbance of the chromophore was read at 535nm against the TBA-TCA-HCl reagent blank using 'Genesys' spectrophotometer.

Values were expressed as mM/100 g wet tissue.

*3.6.1.3. 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

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

**b. Procedure**

1. 3ml 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

   $\Delta t$- Time taken for the decrease in absorbance by 0.05 units (in seconds).

***3.6.1.4. Estimation of Reduced Glutathione***

Reduced glutathione was estimated by the method of Ellman(1959).

**a. Reagents**

1. Phosphate buffer- 0.2M, pH 8.0
2. Trichloro acetic acid : 5 percent
3. Ellman's reagent: 19.8mg of dinitro bis benzoic acid in 100 ml of 1 percent sodium citrate solution.

4. Standard glutathione solution: 10mg of reduced glutathione was dissolved in 100 ml of distilled water.

**b. Procedure**

1. 250 mg of kidney tissue was homogenized with phosphate buffer.
2. From this, 0.5ml was pipetted out and precipitated with 2.0ml of 5 percent trichloro acetic acid.
3. 1.0ml of the supernatant was taken out after centrifugation and 0.5ml of Ellman's reagent and 3.0ml of phosphate buffer were added to it.
4. The yellow colour developed was read at 412nm using 'Genesys' spectrophotometer.
5. A series of standards were treated in a similar manner along with a blank containing 3.5ml of buffer.

The amount of glutathione was expressed as mg/100g of tissue.

### 3.6.2. Estimation of Serum parameters

#### *3.6.2.1. Creatinine*

Creatinine in serum was determined based on Jaffe kinetic method without deproteinisation in 'Genesys' spectrophotometer using Merkotest kit from Merck Specialities Private Limited, India.

Principle:

Creatinine forms a yellow-orange compound in alkaline solution with picric acid. At a low concentration of picric acid as used in this method, precipitation of protein does not take place.

Reagents:

Reagent 1: Buffer solution

Reagent 2: Picric acid

Reagent 3: Creatinine standard, 1 mg/dl

Procedure:

| | Sample/ Standard |
|---|---|
| Sample/Standard | 100µl |
| Buffer solution | 500µl |
| Mixed and incubated for 2 minutes and then added | |
| Picric acid | 500µl |
| Mixed and read absorbance A1 after 60 sec, read absorbance A2 after further 120 sec. | |

Calculation:

Creatinine [mg/dl] = ΔA Sample / ΔA Standard x Concentration of Standard [mg/dl]

*3.6.2.2. Urea*

Urea was estimated according to the Urease GLDH method (kinetic UV test) in semi automatic blood analyzer (Microlab 200) using Ecoline kit from Merck Specialities Private Limited, India.

Principle:

$Urea + 2\ H_2O \text{ ---------> } 2\ NH_4^+ + 2\ HCO_3^-$

$2\text{-Oxoglutarate} + NH_4^+ + NADH \text{ .....> } L\text{-Glutamate} + NAD^+ + H_2O$

Reagents:

Reagent 1: Reagent solution

Reagent 2: Start reagent

Reagent 3: Standard solution

Four volumes of Reagent 1 was mixed with one volume of Reagent 2 and the monoreagent was left for 30 minutes at room temperature.

Procedure:

| | |
|---|---|
| Sample or Standard | 10µl |
| Monoreagent | 1000µl |
| Mixed, incubated for 60 sec. at 25°C and read absorbance A1. After exactly 60sec. read absorbance A2. | |

Calculation:

Urea [mg/dl] = ΔA Sample / ΔA Standard x Concentration of Standard [mg/dl]

*3.6.2.3. Albumin*

Albumin was estimated according to the bromocresol green method in semi automatic blood analyzer (Microlab200) using Ecoline kit from Merck Specialities Private Limited, India.

Principle:

Serum albumin formed yellow-green to green-blue complex at a slightly acidic pH, which was measured.

Reagents:

Reagent 1: Citrate buffer pH 4.2 30mmol/l

Bromocresol green 0.26mmol/l

Reagent 2: Albumin standard 5.0 g/dl

Procedure:

| | Blank | Sample/ Standard |
|---|---|---|
| Sample/ Standard | - | 10µl |
| Distilled water | 10µl | - |
| Citrate buffer | | |
| Bromocresol Green | | |
| reagent | 1000µl | 1000µl |
| Mixed, incubated for 10 minutes at $37^0$C and read the absorbance against reagent blank within 60 minutes. | | |

Calculation:

Albumin [g /dl] = Absorbance of Sample / Absorbance of Standard x Concentration of Standard [g/ dl]

*3.6.2.4. Total protein*

Total protein in serum was estimated by biuret method in 'Genesys' spectrophotometer using Lyphozyme kit from Beacon Diagnostics, India.

Principle:

In alkaline medium, total protein reacted with copper of biuret reagent causing an increase in absorbance. The increase in absorbance, at 546 nm was due to formation of violet coloured complex and it is directly proportional to the concentration of protein present in the sample.

Reagents:

Reagent 1: Biuret reagent

Reagent 2: Protein standard, 6 g/dl

Procedure:

| Reagent | Blank | Standard | Test |
|---|---|---|---|
| Working reagent | 1ml | 1ml | 1ml |
| Standard | - | 10µl | - |
| Sample | - | - | 10µl |
| Mixed, incubated for 5 minutes at room temperature and the optical density was read at 546nm against reagent blank. | | | |

Calculation:

Total protein [g/dl] = Optical density of Sample / Optical density of Standard x 6

*3.6.2.5. Sodium and Potassium*

Sodium and potassium concentrations in serum were determined by flame photometry using 'Systronics flame photometer 128'. The serum samples were diluted with distilled water in the ratio 1:300.

*3.6.2.6. Calcium*

Calcium in serum was estimated by Arsenazo III-colorimetric method in spectrophotometer ('Genesys') using Labkit from Chemelex, S.A., Barcelona.

Principle:

Calcium with Arsenazo III (1,8-Dihydroxy-3,6-disulpho-2,7-naphthalene-bis (azo)-dibenzenearsonic acid), at neutral pH, yielded a blue coloured complex. The intensity of the colour formed is proportional to the calcium concentration in the sample.

Reagents:

Reagent 1: Imidazol Buffer pH 6.5 100mmol/l

Arsenazo III 120mmol/l

Reagent 2: Calcium aqueous primary calibrator 10 mg/dl

Procedure:

| | Blank | Standard | Sample |
|---|---|---|---|
| Arsenazo reagent | 1ml | 1ml | 1ml |
| Standard | - | 10µl | - |
| Sample | - | - | 10µl |
| Mixed and incubated for 2 minutes and read the absorbance against reagent blank at 650nm. | | | |

Calculation:

Calcium [mg/dl] = Absorbance of Sample / Absorbance of Standard x 10(Calibrator concentration)

**3.6.3. Histopathological examination of kidney**

Representative samples of kidney obtained from the dissected animals were fixed in 10 per cent 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.

3.7. STATISTICAL ANALYSIS OF DATA

The results obtained were analysed using analysis of covariance followed by Duncan's multiple range test for comparison between the groups as described by

Snedecor and Cochran (1985). The cytoprotective enzymes were analysed using analysis of variance (ANOVA). The best treatment from each group was selected and they were tested for significance using Student's t test.

# *Results*

# 4. RESULTS

In the present study, analysis of the plant extracts for the active principles and evaluation of the nephroprotective effect of whole plant of *Hygrophila spinosa* and stem bark of *Mangifera indica* were carried out. The results were analysed and presented in tables and figures.

## 4.1. PHYTOCHEMICAL SCREENING

The results are presented in table 1.

### 4.1.1. Steroids

Salkowski test gave a red colour for ethanolic extracts of *Hygrophila spinosa* and *Mangifera indica*, but not for their corresponding aqueous extracts. As per Lieberman Burchardt test, ethanolic extracts gave a reddish ring at the junction which was not seen with the corresponding aqueous extracts. Thus it could be concluded that steroids were present in the ethanolic extracts of *Hygrophila spinosa* and *Mangifera indica*, but no steroids could be detected in the aqueous extracts of *Hygrophila spinosa* and *Mangifera indica*.

### 4.1.2. Alkaloids

No alkaloids were present as per Mayer's test, Wagner's test, Hager's test and Dragendroff's test.

### 4.1.3. Phenolic compounds

Development of blue colour was observed in all the four extracts (ethanolic and aqueous extracts of *Hygrophila spinosa* and ethanolic and aqueous extracts of *Mangifera indica*) which indicted the presence of phenolic compounds.

### 4.1.4. Tannins

Ferric chloride test yielded a bluish brown colour for aqueous extract of *Hygrophila spinosa* and ethanolic and aqueous extracts of *Mangifera indica* but not for ethanolic extract of *Hygrophila spinosa*. White precipitate was developed by mixing a few drops of one per cent gelatin containing ten per cent sodium chloride in aqueous extract of *Hygrophila spinosa* and ethanolic and aqueous extracts of *Mangifera indica* which was not observed in the ethanolic extract of *Hygrophila spinosa*. This revealed the presence of tannins in the aqueous extract of *Hygrophila spinosa* and in the ethanolic and aqueous extracts of *Mangifera indica*.

### 4.1.5. Flavonoids

Ferric chloride and lead acetate tests indicated the presence of flavonoids in ethanolic extracts of *Hygrophila spinosa* and *Mangifera indica* which was not observed in the aqueous extracts of *Hygrophila spinosa* and *Mangifera indica*.

### 4.1.6. Glycosides

As per sodium hydroxide test, yellow colour was observed with the aqueous and ethanolic extracts of *Mangifera indica* but not with the aqueous and ethanolic extracts of *Hygrophila spinosa* indicating the presence of glycosides in the aqueous and ethanolic extracts of *Mangifera indica*. Brown to red colour was developed with the aqueous and ethanolic extracts of *Mangifera indica* which indicated the presence of glycosides in these extracts, but not in the aqueous and ethanolic extracts of *Hygrophila spinosa*.

### 4.1.7. Diterpenes

Diterpenes were present in the ethanolic extract of *Hygrophila spinosa*, ethanolic extract of *Mangifera indica* and aqueous extract of *Mangifera indica* which was indicated by the development of green colour. Aqueous extract of *Hygrophila spinosa* yielded no diterpenes.

**Table 1. Phytochemical screening of the plant extracts**

| Active principles | Ethanolic extract of *Hygrophila spinosa* | Aqueous extract of *Hygrophila spinosa* | Ethanolic extract of *Mangifera indica* | Aqueous extract of *Mangifera indica* |
|---|---|---|---|---|
| Steroids | | | | |
| a) Salkowski test | present | not present | present | not present |
| b) Liberman Burchardt test | present | not present | present | not present |
| Alkaloids | | | | |
| a) Mayer's test | not present | not present | not present | not present |
| b) Wagner's test | not present | not present | not present | not present |
| c) Hager's test | not present | not present | not present | not present |
| d) Dragendroff's test | not present | not present | not present | not present |
| Phenolic compounds | present | present | present | present |
| Tannins | | | | |
| a) Ferric chloride test | not present | present | present | present |
| b) Gelatin test | not present | present | present | present |
| Flavonoids | | | | |
| a) Ferric chloride test | present | not present | present | not present |
| b) Lead acetate test | present | not present | present | not present |
| Glycosides | | | | |
| a) Sodium hydroxide test | not present | not present | present | present |
| b) Benedict's test | not present | not present | present | present |
| Diterpenes | present | not present | present | present |
| Triterpenes | | | | |
| a) Salkowski test | not present | not present | not present | not present |
| b) Liberman Burchardt test | not present | not present | not present | not present |
| Saponins | present | present | present | not present |

### 4.1.8. Triterpenes

Salkowski and Lieberman Burchardt tests indicated the absence of triterpenes in all the four extracts.

### 4.1.9. Saponins

Development of foam that persisted for ten minutes was observed with ethanolic and aqueous extracts of *Hygrophila spinosa* and ethanolic extract of *Mangifera indica* but not with aqueous extract of *Mangifera indica* which indicated the presence of saponins.

## 4.2. OBSERVATIONS

### 4.2.1. Antioxidant enzymes

#### *4.2.1.1. Superoxide dismutase*

The values obtained are presented in Tables 2 and 3 and Fig. 3 and 4. The normal superoxide dismutase level was found to be 2.494 ± 0.12 units/ mg of protein. A significant increase in the level of superoxide dismutase was noted in all the treatment groups when compared with gentamicin control (1.612 ± 0.05 units/ mg of protein). The superoxide dismutase levels following treatment with ethanolic extract of *H. spinosa* at doses 50 mg/kg (Group III) and 250 mg/kg (Group IV) were 4.317 ± 0.08 and 9.676 ± 0.15 units/ mg of protein respectively ($P<0.05$). Similarly, the treatment with aqueous extract of *H. spinosa* at doses 50 mg/kg (Group V) and 250 mg/kg (Group VI) showed significant increase in superoxide dismutase levels with a mean value of 4.209 ± 0.11 and 9.669 ± 0.13 units/ mg of protein respectively ($P<0.05$). The results indicated that Group IV and Group VI produced higher superoxide dismutase levels with the mean value more for Group IV.

Ethanolic extract of *M. indica* at doses 100 mg/kg (Group VII) and 500 mg/kg (Group VIII) showed significant increase in superoxide dismutase levels with mean values of 11.826 ± 0.53 and 20.616 ± 0.69 units/ mg of protein respectively

(Group IX) and 500 mg/kg (Group X), significant increase in superoxide dismutase levels were noted and the mean values were found to be 5.914 ± 0.13 and 10.821 ± 0.33 units/ mg of protein respectively ($P<0.05$). The results indicated that Group VIII and Group X produced higher superoxide dismutase levels and they differ significantly ($P<0.05$) with maximum superoxide level in Group VIII.

*4.2.1.2. Lipid peroxidation*

The results are presented in the Tables 2 and 3 and Fig. 5 and 6. Gentamicin produced significant nephrotoxicity with an increase in lipid peroxidation (36.400 ± 0.95 mM/ 100 mg of tissue). The normal lipid peroxidation level in the kidney tissue was found to be 24.367 ± 0.43 mM/ 100 mg of tissue. The treatment with ethanolic extract of *H. spinosa* at a dose rate of 50 mg/kg (Group III) indicated a lipid peroxidation value of 36.342 ± 0.96 mM/ 100 mg of tissue which does not differ significantly from gentamicin alone treated group (Group II) ($P>0.05$). But ethanolic extract of *H. spinosa* at a dose rate of 250 mg/kg (Group IV) showed a significant decrease in lipid peroxidation (14.716 ± 0.60 mM/ 100 mg of tissue). Treatment with aqueous extract of *H. spinosa* at doses 50 mg/kg (Group V) and 250 mg/kg (Group VI) showed lipid peroxidation levels of 32.813 ± 0.87 and 22.213 ± 1.00 mM/ 100 mg of tissue (values were comparable with the normal value) respectively. The results indicated that Group IV and Group VI produced maximum significant reduction in lipid peroxidation when compared with other treatment groups and they differ significantly ($P<0.05$) with a mean value less for Group IV.

Similarly, ethanolic extract of *M. indica* at doses 100 mg/kg (Group VII) and 500 mg/kg (Group VIII) produced significant reduction in lipid peroxidation with mean values of 22.138 ± 0.47 and 17.600 ± 0.89 mM/ 100 mg of tissue respectively ($P<0.05$) while their corresponding aqueous extracts produced significant reduction with mean values of 15.912 ± 0.45 (Group IX) and 9.688 ± 0.87 (Group X) mM/ 100 mg of tissue respectively ($P<0.05$). The results indicated that Group VIII and Group

mg of tissue respectively (P<0.05). The results indicated that Group VIII and Group X produced maximum reduction in lipid peroxidation when compared with other treatment groups and they differ significantly (P<0.05) with a lesser mean value for Group X.

*4.2.1.3. Catalase*

The results are shown in Tables 2 and 3 and Fig. 7 and 8. An increase in catalase levels were noted in Groups III, IV, V and VI when compared with Group II (P<0.05). The normal catalase level was found to be 0.988 ± 0.02 units/ assay mixture. After 30 days of experiment, the catalase levels in Groups III, IV, V and VI were found to be 1.932 ± 0.01, 4.581 ± 0.01, 2.058 ± 0.01 and 3.999 ± 0.01 units/ assay mixture respectively and all the groups significantly differed from Group II (P<0.05). Group II showed a catalase level of 0.722 ± 0.01 units/ assay mixture. The results indicated that Group IV and Group VI produced higher catalase levels with a maximum catalase level in Group IV.

Similarly, the catalase levels in Groups VII, VIII, IX and X (*M. indica* treated groups) were found to be 2.278 ± 0.01, 4.883 ± 0.01, 2.964 ± 0.02 and 5.543 ± 0.03 units/ assay mixture respectively and all the groups significantly differed with each other and also from Group II (P<0.05). Higher catalase values were shown by Group VIII and Group X with a maximum catalase value in Group X.

*4.2.1.4. Reduced glutathione*

The results are given in Tables 2 and 3 and Fig. 9 and 10. The normal reduced glutathione level was found to be 133.525 ± 3.23 mg/ 100g of tissue. All the treatment groups showed significant increase in reduced glutathione levels when compared with Group II (81.337 ± 2.97) (P<0.05). Group III and Group IV showed higher reduced glutathione levels with mean values of 249.350 ± 1.90 and 289.037 ± 2.89 mg/ 100g of tissue respectively while Group V and Group VI produced mean values of 198.438 ± 1.72 and 171.262 ± 1.96 mg/ 100g of tissue respectively. The results indicated that Group IV and Group V showed higher reduced glutathione

**Table 2. Effect of ethanolic and aqueous extracts of *Hygrophila spinosa* on superoxide dismutase, lipid peroxidation, catalase and reduced glutathione levels**

| Group | Superoxide dismutase levels of kidney tissue, units / mg of protein (Mean ± SE) | Lipid peroxidation in kidney tissue, mM /100 mg of tissue (Mean ± SE) | Catalase levels of kidney tissue, units / assay mixture (250 mg) (Mean ± SE) | Reduced glutathione levels of kidney tissue, mg / 100g of tissue (Mean ± SE) |
|---|---|---|---|---|
| I | $2.494 \pm 0.12^{c}$ | $24.367 \pm 0.43^{d}$ | $0.988 \pm 0.02^{e}$ | $133.525 \pm 3.23^{e}$ |
| II | $1.612 \pm 0.05^{d}$ | $36.400 \pm 0.95^{b}$ | $0.722 \pm 0.01^{f}$ | $81.337 \pm 2.97^{f}$ |
| III | $4.317 \pm 0.08^{b}$ | $36.342 \pm 0.96^{b}$ | $1.932 \pm 0.01^{d}$ | $249.350 \pm 1.90^{b}$ |
| IV | $9.676 \pm 0.15^{a}$ | $14.716 \pm 0.60^{e}$ | $4.581 \pm 0.01^{a}$ | $289.037 \pm 2.89^{a}$ |
| V | $4.209 \pm 0.11^{b}$ | $32.813 \pm 0.87^{c}$ | $2.058 \pm 0.01^{c}$ | $198.438 \pm 1.72^{c}$ |
| VI | $9.669 \pm 0.13^{a}$ | $22.213 \pm 1.00^{d}$ | $3.999 \pm 0.01^{b}$ | $171.262 \pm 1.96^{d}$ |

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

**Table 3. Effect of ethanolic and aqueous extracts of *Mangifera indica* on superoxide dismutase, lipid peroxidation, catalase and reduced glutathione levels**

| Group | Superoxide dismutase levels of kidney tissue, units / mg of protein (Mean ± SE) | Lipid peroxidation in kidney tissue, mM /100 mg of tissue (Mean ± SE) | Catalase levels of kidney tissue, units / assay mixture (250 mg) (Mean ± SE) | Reduced glutathione levels of kidney tissue, mg / 100g of tissue (Mean ± SE) |
|---|---|---|---|---|
| I | $2.494 \pm 0.12^{d}$ | $24.367 \pm 0.43^{b}$ | $0.988 \pm 0.02^{e}$ | $133.525 \pm 3.23^{d}$ |
| II | $1.612 \pm 0.05^{d}$ | $36.400 \pm 0.95^{a}$ | $0.722 \pm 0.01^{f}$ | $81.337 \pm 2.97^{e}$ |
| VII | $11.826 \pm 0.53^{b}$ | $22.138 \pm 0.47^{c}$ | $2.278 \pm 0.01^{d}$ | $159.672 \pm 3.68^{c}$ |
| VIII | $20.616 \pm 0.69^{a}$ | $17.600 \pm 0.89^{d}$ | $4.883 \pm 0.01^{b}$ | $229.212 \pm 3.45^{a}$ |
| IX | $5.914 \pm 0.13^{c}$ | $15.912 \pm 0.45^{d}$ | $2.964 \pm 0.02^{c}$ | $183.825 \pm 2.70^{b}$ |
| X | $10.821 \pm 0.33^{b}$ | $9.688 \pm 0.87^{e}$ | $5.543 \pm 0.03^{a}$ | $221.487 \pm 3.75^{a}$ |

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

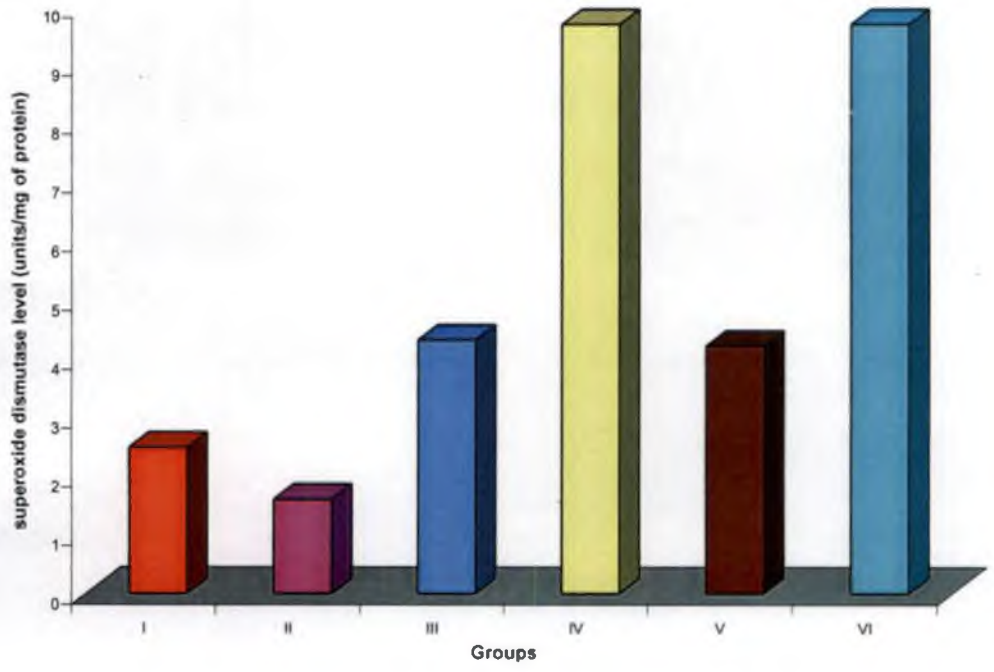


**Fig.3. Effect of ethanolic and aqueous extracts of *H. spinosa* on superoxide dismutase levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group III** – ethanolic extract *H. spinosa* 50 mg/kg treated group
**Group IV** – ethanolic extract *H. spinosa* 250 mg/kg treated group
**Group V** – aqueous extract *H. spinosa* 50 mg/kg treated group
**Group VI** – aqueous extract *H. spinosa* 250 mg/kg treated group

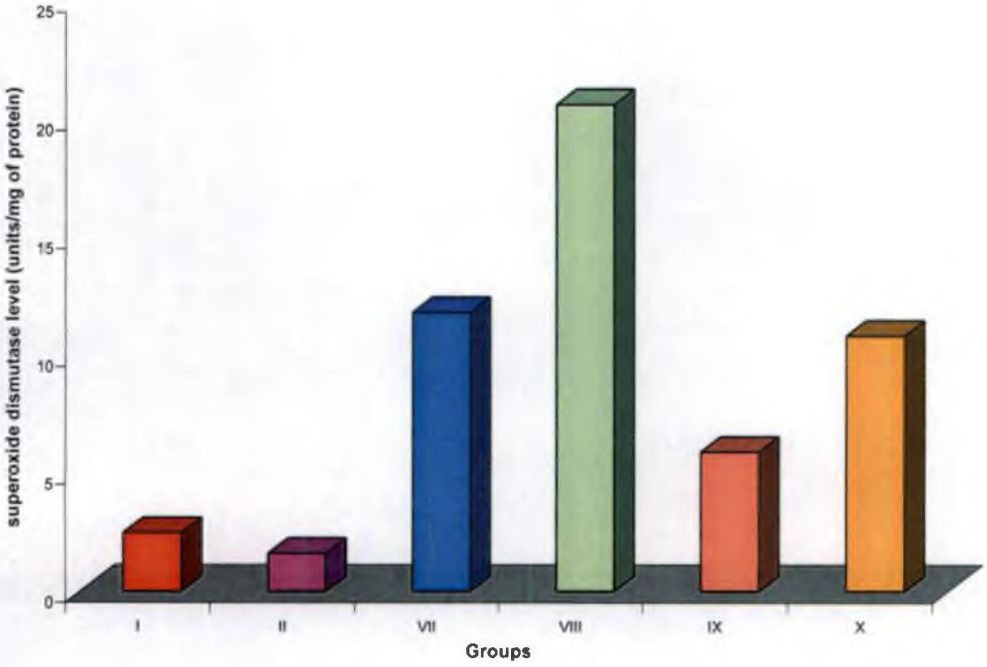


**Fig.4. Effect of ethanolic and aqueous extracts of *M. indica* on superoxide dismutase levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group VII** – ethanolic extract *M. indica* 100 mg/kg treated group
**Group VIII** – ethanolic extract *M. indica* 500 mg/kg treated group
**Group IX** – aqueous extract *M. indica* 100 mg/kg treated group
**Group X** – aqueous extract *M. indica* 500 mg/kg treated group

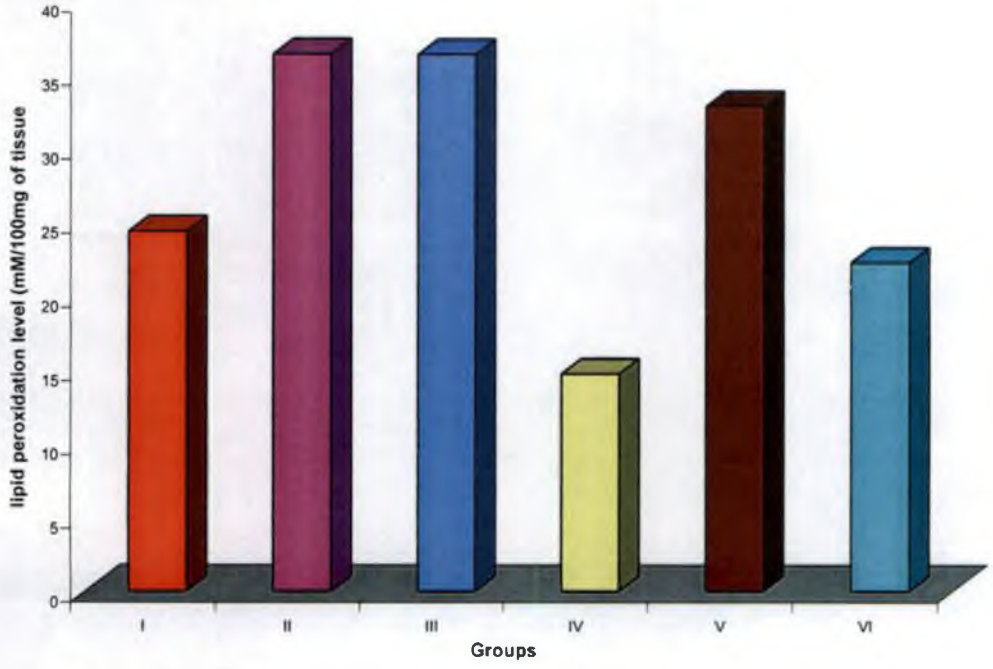


**Fig.5. Effect of ethanolic and aqueous extracts of *H. spinosa* on lipid peroxidation levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group III** – ethanolic extract *H. spinosa* 50 mg/kg treated group
**Group IV** – ethanolic extract *H. spinosa* 250 mg/kg treated group
**Group V** – aqueous extract *H. spinosa* 50 mg/kg treated group
**Group VI** – aqueous extract *H. spinosa* 250 mg/kg treated group

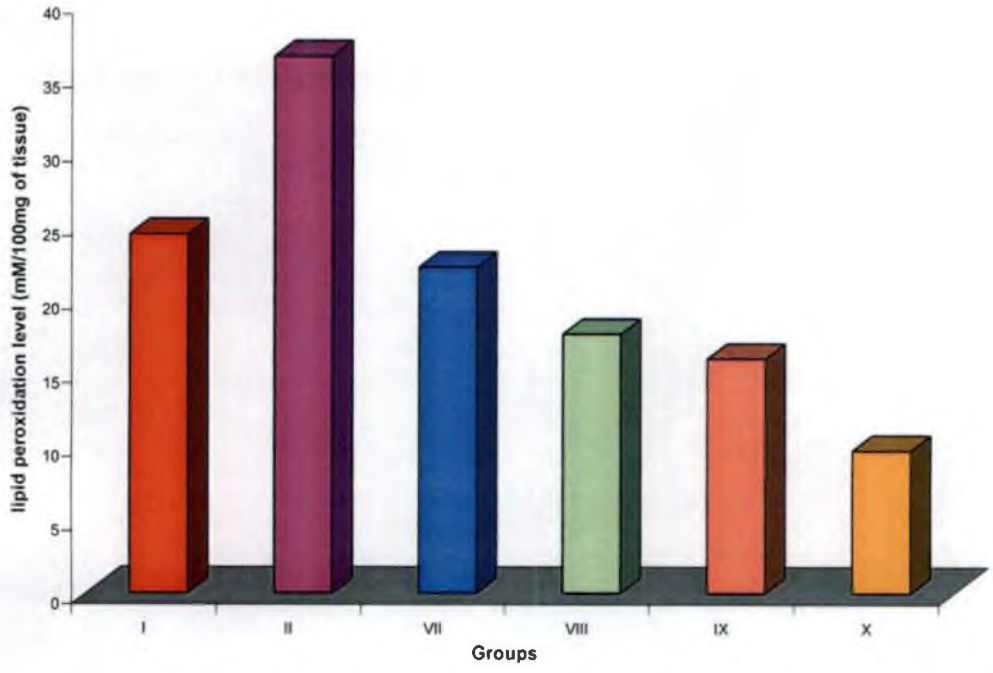


**Fig.6. Effect of ethanolic and aqueous extracts of *M. indica* on lipid peroxidation levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group VII** – ethanolic extract *M. indica* 100 mg/kg treated group
**Group VIII** – ethanolic extract *M. indica* 500 mg/kg treated group
**Group IX** – aqueous extract *M. indica* 100 mg/kg treated group
**Group X** – aqueous extract *M. indica* 500 mg/kg treated group

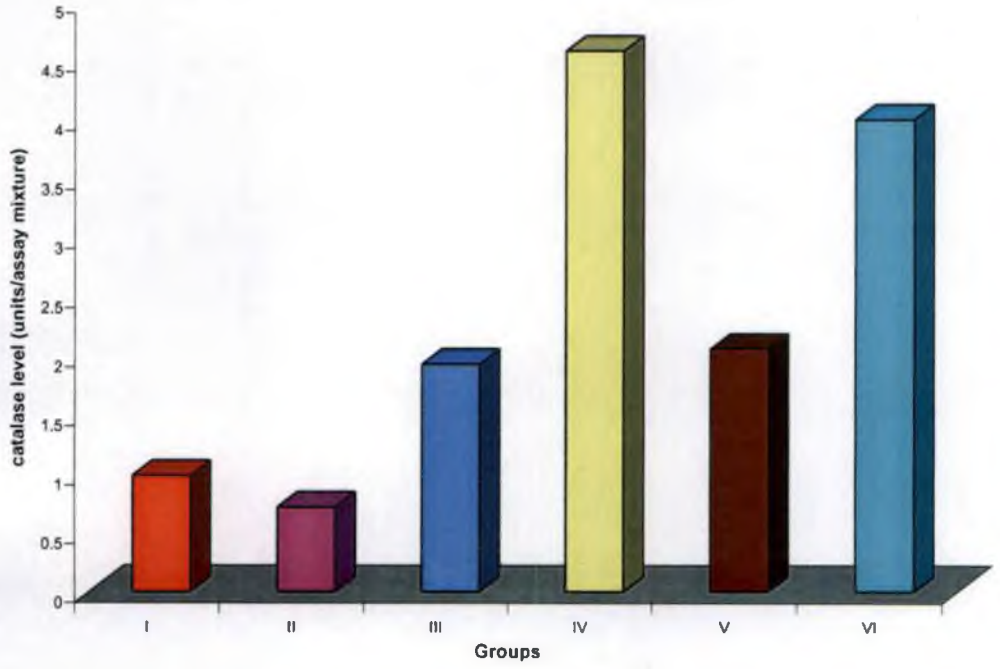


**Fig.7. Effect of ethanolic and aqueous extracts of *H. spinosa* on catalase**

**Group I** – normal group
**Group II** – gentamicin group
**Group III** – ethanolic extract *H. spinosa* 50 mg/kg treated group
**Group IV** – ethanolic extract *H. spinosa* 250 mg/kg treated group
**Group V** – aqueous extract *H. spinosa* 50 mg/kg treated group
**Group VI** – aqueous extract *H. spinosa* 250 mg/kg treated group

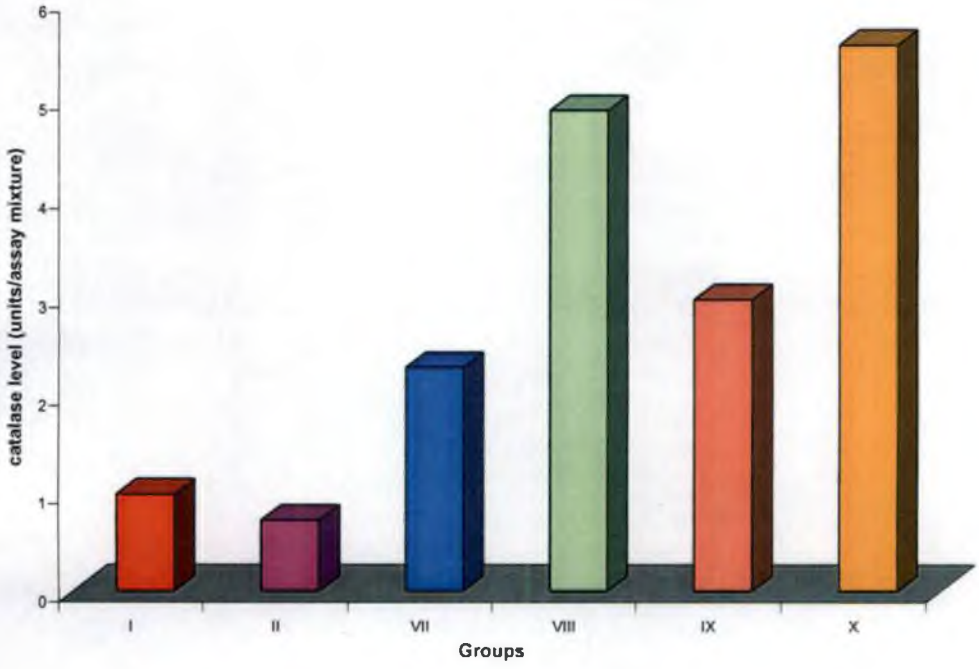


**Fig.8. Effect of ethanolic and aqueous extracts of *M. indica* on catalase**

**Group I** – normal group
**Group II** – gentamicin group
**Group VII** – ethanolic extract *M. indica* 100 mg/kg treated group
**Group VIII** – ethanolic extract *M. indica* 500 mg/kg treated group
**Group IX** – aqueous extract *M. indica* 100 mg/kg treated group
**Group X** – aqueous extract *M. indica* 500 mg/kg treated group

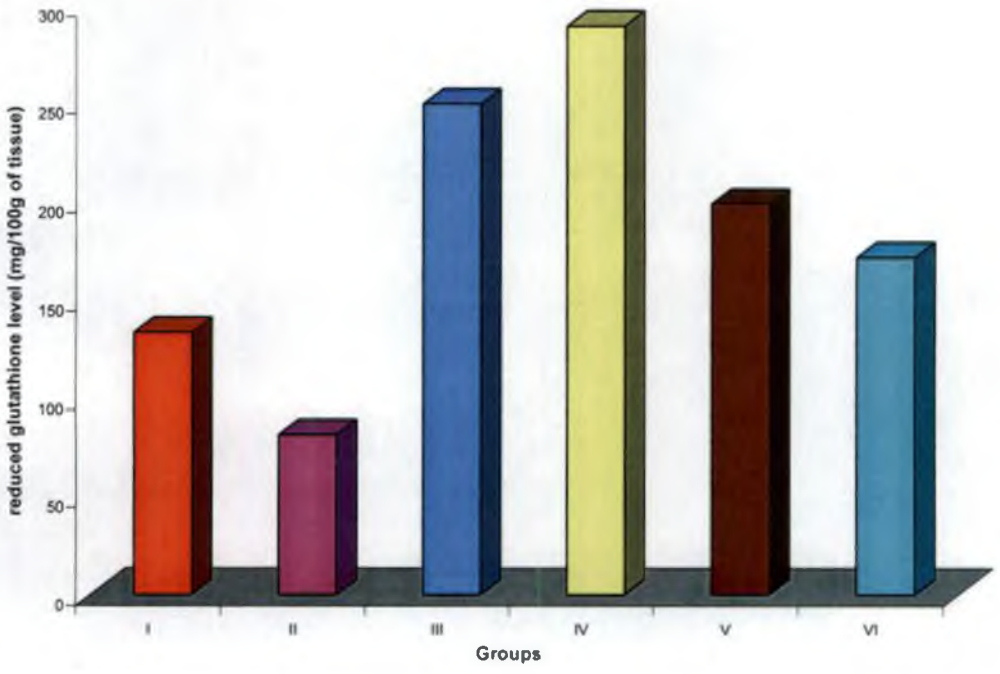


**Fig.9. Effect of ethanolic and aqueous extracts of *H. spinosa* on reduced glutathione levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group III** – ethanolic extract *H. spinosa* 50 mg/kg treated group
**Group IV** – ethanolic extract *H. spinosa* 250 mg/kg treated group
**Group V** – aqueous extract *H. spinosa* 50 mg/kg treated group
**Group VI** – aqueous extract *H. spinosa* 250 mg/kg treated group

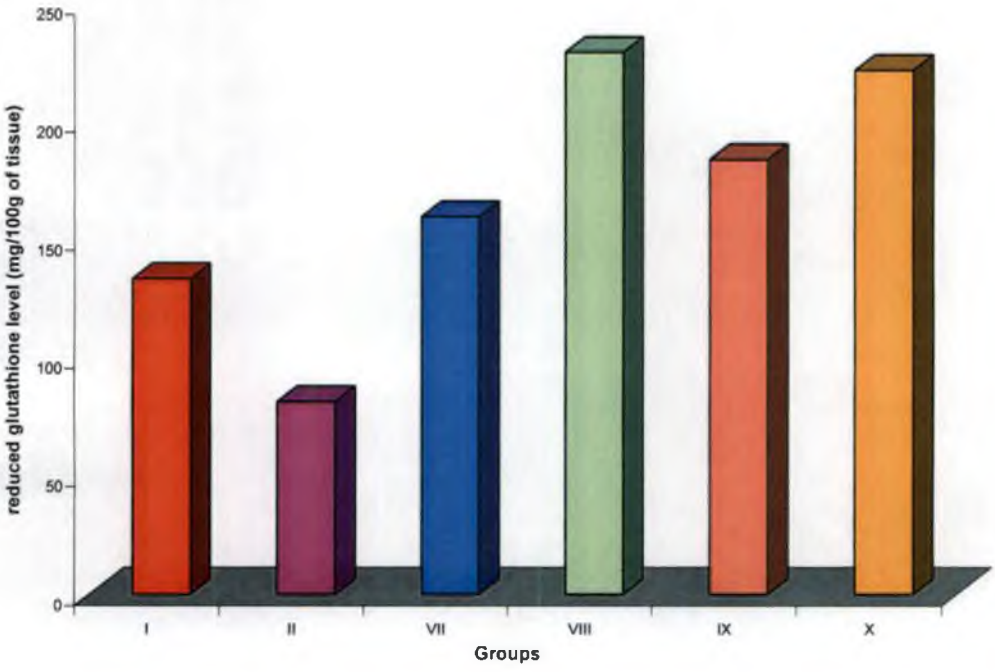


**Fig.10. Effect of ethanolic and aqueous extracts of *M. indica* on reduced glutathione levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group VII** – ethanolic extract *M. indica* 100 mg/kg treated group
**Group VIII** – ethanolic extract *M. indica* 500 mg/kg treated group
**Group IX** – aqueous extract *M. indica* 100 mg/kg treated group
**Group X** – aqueous extract *M. indica* 500 mg/kg treated group

levels and they differ significantly (P<0.05) with Group IV showing the maximum reduced glutathione level.

Similarly, among *M. indica* treated groups, Group VIII and Group X produced maximum reduced glutathione levels with mean values of 229.212 ± 3.45 and 221.487 ± 3.75 mg/ 100g of tissue respectively and they do not differ significantly (P>0.05). The reduced glutathione levels of Group VII and Group IX were 159.672 ± 3.68 and 183.825 ± 2.70 mg/ 100g of tissue respectively. The mean value being higher for Group VIII, it showed maximum reduced glutathione level.

### 4.2.2. Serum parameters

#### *4.2.2.1. Creatinine*

The results of the effect of ethanolic and aqueous extracts of *H. spinosa* on serum creatinine levels are presented in Table 4 and Fig. 11. On $0^{th}$ day, the mean serum creatinine values of Groups I to VI were 0.54 ± 0.05, 0.45 ± 0.03, 0.47 ± 0.03, 0.48 ± 0.03, 0.46 ± 0.03 and 0.44 ± 0.03 mg/dl respectively.

On $9^{th}$ day, all the groups except the normal group showed an increase in serum creatinine values with mean values of 0.67 ± 0.03, 2.81 ± 0.36, 2.84 ± 0.27, 3.90 ± 0.39, 1.96 ± 0.15 and 2.44 ± 0.17 mg/dl respectively for Groups I, II, III, IV, V and VI.

On $15^{th}$ day, there was a significant reduction in serum creatinine values in Groups III to VI when compared with Group II (P<0.05). The creatinine values of Group IV and Group VI were almost comparable to healthy control with mean creatinine values of 0.51 ± 0.05 and 0.56 ± 0.08 mg/dl respectively. The serum creatinine values of Group I, II, III and V were 0.52 ± 0.04, 2.13 ± 0.23, 1.70 ± 0.10, 1.20 ± 0.20 mg/dl respectively.

On $30^{th}$ day, all the treatment groups showed a significant reduction in serum creatinine values when compared with Group II (1.56 ± 0.18). The serum creatinine

values of Groups I, IV, V and VI were comparable with mean values of 0.58 ± 0.05, 0.45 ± 0.05, 0.64 ± 0.03, 0.57 ± 0.05 mg/dl respectively. The mean serum creatinine value of group III was 1.03 ± 0.14 mg/dl which was less than Group II (P>0.05).

The results indicated that ethanolic and aqueous extracts of *H. spinosa* at a dose rate of 250 mg/kg (Groups IV and VI) showed considerable reduction in serum creatinine values than the other treatment groups. Of this, ethanolic extract of *H. spinosa* at a dose rate of 250 mg/kg (Group IV) showed maximum reduction in serum creatinine values by comparing the mean values.

The results of the effect of ethanolic and aqueous extracts of *M. indica* on serum creatinine levels are presented in Table 5 and Fig. 12. On $0^{th}$ day, the mean serum creatinine values of Groups I, II, VII, VIII, IX and X were 0.54 ± 0.05, 0.45 ± 0.03, 0.44 ± 0.03, 0.45 ± 0.03, 0.44 ± 0.04 and 0.44 ± 0.06 mg/dl respectively.

On $9^{th}$ day, all the groups except the normal group showed an increase in serum creatinine values with mean values of 0.67 ± 0.03, 2.81 ± 0.36, 2.63 ± 0.16, 2.21 ± 0.21, 2.60 ± 0.12, 2.06 ± 0.17 mg/dl respectively for Groups I, II, VII, VIII, IX and X. The serum creatinine values of Groups VII, VIII and IX were comparable (P>0.05).

On $15^{th}$ day, there was a reduction in serum creatinine values in Groups VII to X when compared with Group II (P<0.05). The serum creatinine values of Groups VII, IX and X were comparable with mean values of 0.92 ± 0.02, 0.89 ± 0.02 and 0.88 ± 0.03 mg/dl respectively while Groups I and VIII had comparable values (0.52 ± 0.04 and 0.59 ± 0.07 mg/dl). Group II had a serum creatinine value of 2.13 ± 0.23 mg/dl.

On $30^{th}$ day, all the treatment groups showed a significant reduction in serum creatinine values when compared with Group II (1.56 ± 0.18). The serum creatinine values of Groups I, VII, VIII, IX and X were comparable (P> 0.05) with mean values of 0.58 ± 0.05, 0.63 ± 0.04, 0.59 ± 0.06, 0.62 ± 0.05, 0.61 ± 0.05 mg/dl respectively.

**Table 4. Effect of ethanolic and aqueous extracts of *Hygrophila spinosa* on serum creatinine levels, mg/dl**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 0.54 ± 0.05 | $0.67 \pm 0.03^{d}$ | $0.52 \pm 0.04^{d}$ | $0.58 \pm 0.05^{c}$ |
| II | 0.45 ± 0.03 | $2.81 \pm 0.36^{b}$ | $2.13 \pm 0.23^{a}$ | $1.56 \pm 0.18^{a}$ |
| III | 0.47 ± 0.03 | $2.84 \pm 0.27^{b}$ | $1.70 \pm 0.10^{b}$ | $1.03 \pm 0.14^{b}$ |
| IV | 0.48 ± 0.03 | $3.90 \pm 0.39^{a}$ | $0.51 \pm 0.05^{d}$ | $0.45 \pm 0.05^{c}$ |
| V | 0.46 ± 0.03 | $1.96 \pm 0.15^{c}$ | $1.20 \pm 0.20^{c}$ | $0.64 \pm 0.03^{c}$ |
| VI | 0.44 ± 0.03 | $2.44 \pm 0.17^{bc}$ | $0.56 \pm 0.08^{d}$ | $0.57 \pm 0.05^{c}$ |

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

**Table 5. Effect of ethanolic and aqueous extracts of *Mangifera indica* on serum creatinine levels, mg/dl**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 0.54 ± 0.05 | $0.67 \pm 0.03^{c}$ | $0.52 \pm 0.04^{c}$ | $0.58 \pm 0.05^{b}$ |
| II | 0.45 ± 0.03 | $2.81 \pm 0.36^{a}$ | $2.13 \pm 0.23^{a}$ | $1.56 \pm 0.18^{a}$ |
| VII | 0.44 ± 0.03 | $2.63 \pm 0.16^{ab}$ | $0.92 \pm 0.02^{b}$ | $0.63 \pm 0.04^{b}$ |
| VIII | 0.45 ± 0.03 | $2.21 \pm 0.21^{ab}$ | $0.59 \pm 0.07^{c}$ | $0.59 \pm 0.06^{b}$ |
| IX | 0.44 ± 0.04 | $2.60 \pm 0.12^{ab}$ | $0.89 \pm 0.02^{b}$ | $0.62 \pm 0.05^{b}$ |
| X | 0.44 ± 0.06 | $2.06 \pm 0.17^{b}$ | $0.88 \pm 0.03^{b}$ | $0.61 \pm 0.05^{b}$ |

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

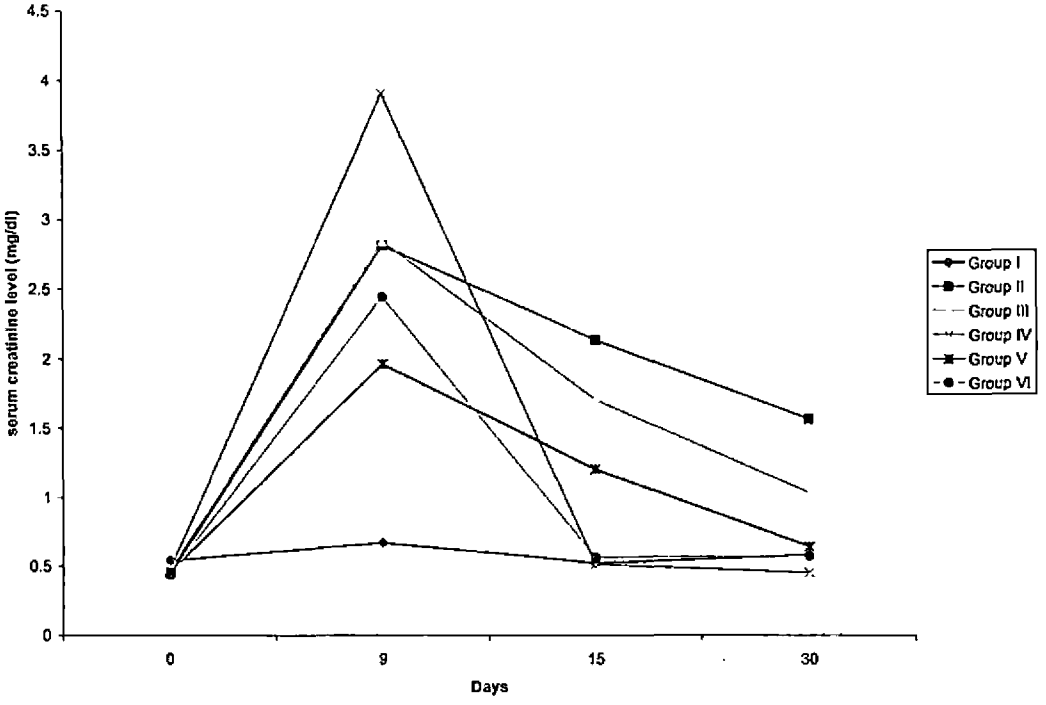


**Fig.11. Effect of ethanolic and aqueous extracts of *H. spinosa* on serum creatinine levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group III** – ethanolic extract *H. spinosa* 50 mg/kg treated group
**Group IV** – ethanolic extract *H. spinosa* 250 mg/kg treated group
**Group V** – aqueous extract *H. spinosa* 50 mg/kg treated group
**Group VI** – aqueous extract *H. spinosa* 250 mg/kg treated group

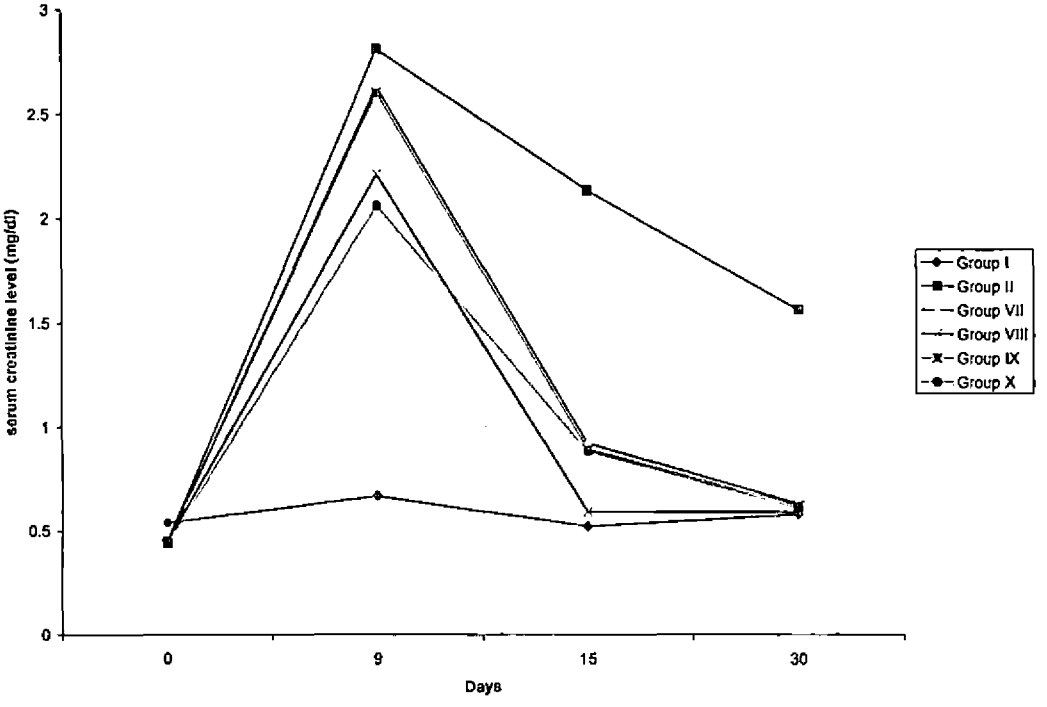


**Fig.12. Effect of ethanolic and aqueous extracts of *M. indica* on serum creatinine levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group VII** – ethanolic extract *M. indica* 100 mg/kg treated group
**Group VIII** – ethanolic extract *M. indica* 500 mg/kg treated group
**Group IX** – aqueous extract *M. indica* 100 mg/kg treated group
**Group X** – aqueous extract *M. indica* 500 mg/kg treated group

The results indicated that ethanolic and aqueous extracts of *M. indica* at a dose rate of 500 mg/kg (Groups VIII and X) showed considerable reduction in serum creatinine values than the other treatment groups. Of this, ethanolic extract of *M. indica* at a dose rate of 500 mg/kg (Group VIII) showed maximum reduction in serum creatinine values by comparing the mean values.

*4.2.2.2. Urea*

The results of the effect of ethanolic and aqueous extracts of *H. spinosa* on serum urea levels are presented in Table 6 and the time course of serum urea levels is displayed in Fig. 13. On $0^{th}$ day, the mean serum urea levels were 46.88 ± 1.90, 28.63 ± 1.92, 33.38 ± 1.28, 31.63 ± 1.97, 30.50 ± 3.50 and 31.38 ± 1.08 mg/dl for Groups I, II, III, IV V and VI respectively.

On $9^{th}$ day, all the groups except the normal group showed an increase in serum urea levels with mean values of 50.25 ± 2.02, 125.63 ± 4.74, 123.63 ± 3.87, 154.63 ± 2.21, 69.50 ± 2.95 and 77.38 ± 2.09 mg/dl respectively for Groups I to VI. Groups II and III did not differ significantly while significant difference was shown by Group IV. Groups V and VI also did not differ significantly.

On $15^{th}$ day, there was a significant reduction in serum urea levels in the treatment groups when compared with Group II (67.63 ± 2.62 mg/dl) except in Group III where the serum urea level was comparable with Group II. The serum urea levels of Groups IV, V and VI did not differ significantly ($P>0.05$) with mean values of 52.88 ± 1.56, 51.63 ± 2.69 and 53.63 ± 2.86 mg/dl respectively.

On $30^{th}$ day, significant reduction in the mean serum urea levels of all the treated groups were noted ($P<0.05$) when compared with Group II (56.63 ± 2.54 mg/dl). The mean serum urea level of Group III was found to be 38.50 ± 2.59 mg/dl while that of Groups IV and VI were 28.13 ± 2.39 and 30.00 ± 1.52 mg/dl respectively. Group V had an intermediate serum urea level of 31.38 ± 1.96 mg/dl.

The results indicated that the ethanolic and aqueous extracts of *H. spinosa* at a dose rate of 250 mg/kg (Groups IV and VI) produced significant reduction in serum urea levels when compared with other treatment groups. Of this, the maximum reduction in serum urea levels was shown by ethanolic extract of *H. spinosa* at a dose rate of 250 mg/kg (Group IV) by comparing the mean values.

The results of the effect of ethanolic and aqueous extracts of *M. indica* on serum urea levels are presented in Table 7 and the time course of serum urea levels is shown in Fig. 14. On $0^{th}$ day, the mean serum urea levels were 46.88 ± 1.90, 28.63 ± 1.92, 27.88 ± 1.22, 29.50 ± 0.91, 31.88 ± 1.88, 33.25 ± 1.89 mg/dl for Groups I, II, VII, VIII, IX and X respectively.

On $9^{th}$ day, all the groups except the normal group showed an increase in serum urea levels with mean values of 50.25 ± 2.02, 125.63 ± 4.74, 105.00 ± 2.21, 106.63 ± 2.83, 166.25 ± 2.99, 83.75 ± 1.94 mg/dl respectively for Groups I, II, VII, VIII, IX and X. Groups VII and VIII did not differ significantly while significant difference was shown by Groups IX and X.

On $15^{th}$ day, there was a reduction in serum urea levels when compared with Group II (67.63 ± 2.62 mg/dl). All the groups differed significantly with mean values of 58.50 ± 2.35, 34.13 ± 2.33, 44.87 ± 2.34 mg/dl for Groups VII, VIII and X respectively. The serum urea level of Group IX was intermediate with a mean value of 41.75 ± 2.27 mg/dl.

On $30^{th}$ day, significant reduction in the mean serum urea levels of all the treated groups were noted ($P<0.05$) when compared with Group II (56.63 ± 2.54 mg/dl). The mean serum urea level of Group VII was found to be 53.13 ± 1.98 mg/dl while that of Groups IX and X were comparable, with serum urea values of 45.50 ± 2.51 and 43.63 ± 1.59 mg/dl respectively. Group VIII had a serum urea level of 48.13 ± 1.39 mg/dl.

**Table 6. Effect of ethanolic and aqueous extracts of *Hygrophila spinosa* on serum urea levels, mg/dl**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 46.88 ± 1.90 | $50.25 \pm 2.02^{d}$ | $48.50 \pm 2.81^{b}$ | $50.38 \pm 1.73^{b}$ |
| II | 28.63 ± 1.92 | $125.63 \pm 4.74^{b}$ | $67.63 \pm 2.62^{a}$ | $56.63 \pm 2.54^{a}$ |
| III | 33.38 ± 1.28 | $123.63 \pm 3.87^{b}$ | $74.63 \pm 2.98^{a}$ | $38.50 \pm 2.59^{c}$ |
| IV | 31.63 ± 1.97 | $154.63 \pm 2.21^{a}$ | $52.88 \pm 1.56^{b}$ | $28.13 \pm 2.39^{d}$ |
| V | 30.50 ± 3.50 | $69.50 \pm 2.95^{c}$ | $51.63 \pm 2.69^{b}$ | $31.38 \pm 1.96^{cd}$ |
| VI | 31.38 ± 1.08 | $77.38 \pm 2.09^{c}$ | $53.63 \pm 2.86^{b}$ | $30.00 \pm 1.52^{c}$ |

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

**Table 7. Effect of ethanolic and aqueous extracts of *Mangifera indica* on serum urea levels, mg/dl**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 46.88 ± 1.90 | $50.25 \pm 2.02^{e}$ | $48.50 \pm 2.81^{c}$ | $50.38 \pm 1.73^{d}$ |
| II | 28.63 ± 1.92 | $125.63 \pm 4.74^{b}$ | $67.63 \pm 2.62^{a}$ | $56.63 \pm 2.54^{b}$ |
| VII | 27.88 ± 1.22 | $105.00 \pm 2.21^{c}$ | $58.50 \pm 2.35^{b}$ | $53.13 \pm 1.98^{a}$ |
| VIII | 29.50 ± 0.91 | $106.63 \pm 2.83^{c}$ | $34.13 \pm 2.33^{d}$ | $48.13 \pm 1.39^{c}$ |
| IX | 31.88 ± 1.88 | $166.25 \pm 2.99^{a}$ | $41.75 \pm 2.27^{cd}$ | $45.50 \pm 2.51^{cd}$ |
| X | 33.25 ± 1.89 | $83.75 \pm 1.94^{d}$ | $44.87 \pm 2.34^{c}$ | $43.63 \pm 1.59^{cd}$ |

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

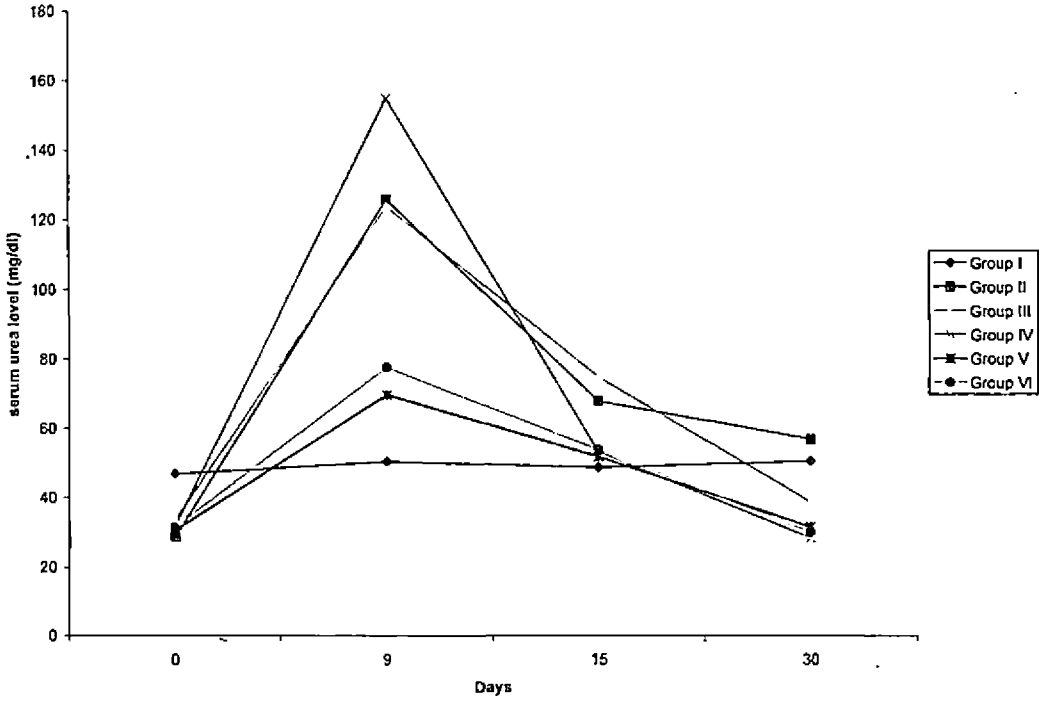


**Fig.13. Effect of ethanolic and aqueous extracts of *H. spinosa* on serum urea levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group III** – ethanolic extract *H. spinosa* 50 mg/kg treated group
**Group IV** – ethanolic extract *H. spinosa* 250 mg/kg treated group
**Group V** – aqueous extract *H. spinosa* 50 mg/kg treated group
**Group VI** – aqueous extract *H. spinosa* 250 mg/kg treated group

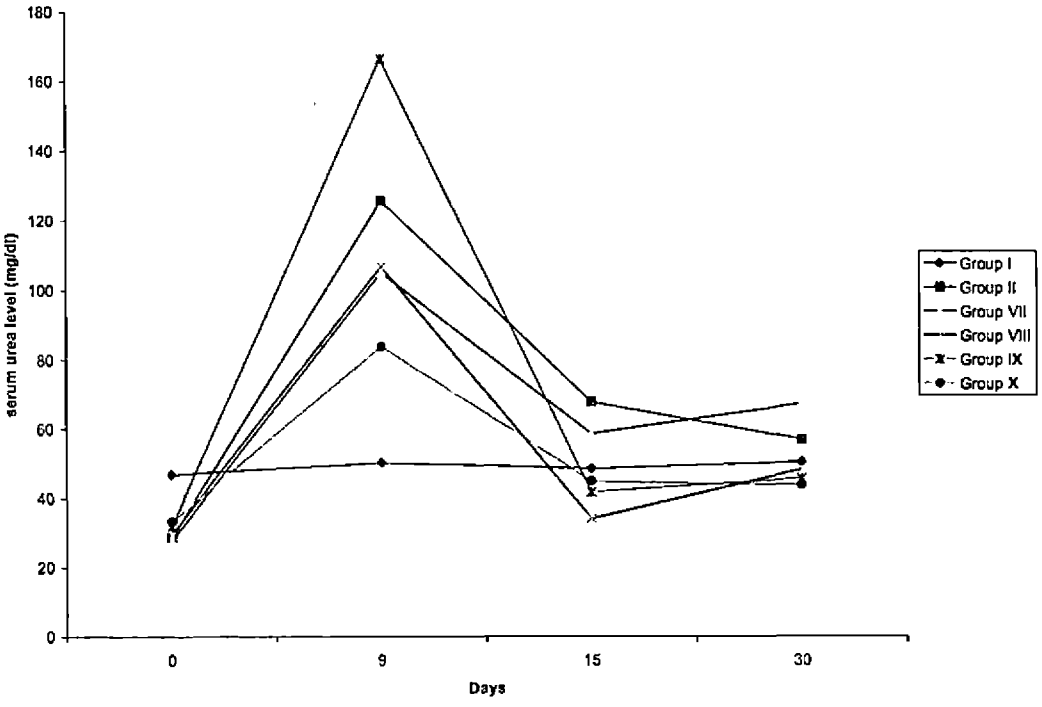


**Fig.14. Effect of ethanolic and aqueous extracts of *M. indica* on serum urea levels**

**Group I** – normal group
**Group II** – gentamicin group
**Group VII** – ethanolic extract *M. indica* 100 mg/kg treated group
**Group VIII** – ethanolic extract *M. indica* 500 mg/kg treated group
**Group IX** – aqueous extract *M. indica* 100 mg/kg treated group
**Group X** – aqueous extract *M. indica* 500 mg/kg treated group

The results indicated that the ethanolic and aqueous extracts of *M. indica* at a dose rate of 500 mg/kg (Groups VIII and X) produced significant reduction in serum urea levels when compared with other treatment groups. Of this, the maximum reduction in serum urea levels was shown by aqueous extract of *M. indica* at a dose rate of 500 mg/kg (Group X) by comparing the mean values.

***4.2.2.3. Albumin***

The results of the effect of ethanolic and aqueous extracts of *H. spinosa* on serum albumin levels are presented in Table 8. On $0^{th}$ day, the mean serum albumin levels of Groups I to IV were 3.96 ± 0.29, 4.35 ± 0.09, 4.26 ± 0.11, 3.96 ± 0.13, 4.08 ± 0.12 and 4.09 ± 0.14 g/dl respectively. On $9^{th}$ day, Group II showed a serum albumin value of 4.15 ± 0.15 g/dl. Groups I and VI had mean values of 3.80 ± 0.22 and 3.90 ± 0.18 g/dl respectively (P>0.05). The mean values of Group III and Group IV were 3.43 ± 0.27 and 3.38 ± 0.13 g/dl respectively (P>0.05). On $15^{th}$ day, the mean serum albumin values of Group II (4.10 ± 0.23 g/dl) and Group IV (3.86 ± 0.19 g/dl) were comparable (P>0.05) while that of Group I and Group VI were 4.58 ± 0.22 and 4.31 ± 0.23 g/dl (P>0.05) respectively. The mean values of Group III and Group V were 3.09 ± 0.30 and 3.48 ± 0.15 g/dl respectively (P>0.05). On $30^{th}$ day, the mean values were 3.73 ± 0.15, 4.55 ± 0.06, 3.44 ± 0.21, 3.84 ± 0.08, 4.21 ± 0.15, 4.76 ± 0.20 respectively for Groups I to VI. The results indicated that the serum albumin levels were within the normal range in all the groups throughout the experiment.

The results of the effect of ethanolic and aqueous extracts of *M. indica* on serum albumin levels are presented in Table 9. On $0^{th}$ day, the mean serum albumin levels of Groups VII to X were 4.07 ± 0.15, 4.06 ± 0.16, 4.00 ± 0.14 and 4.23 ± 0.19 g/dl respectively. On $9^{th}$ day, Groups I, II, VIII and IX showed mean values of 3.80 ± 0.22, 4.15 ± 0.15, 3.75 ± 0.17, 3.55 ± 0.09 g/dl respectively which did not differ

**Table 8. Effect of ethanolic and aqueous extracts of *Hygrophila spinosa* on serum albumin levels, g/dl**

| Group | 0th day (Mean ± SE) | 9th day (Mean ± SE) | 15th day (Mean ± SE) | 30th day (Mean ± SE) |
|---|---|---|---|---|
| I | 3.96 ± 0.29 | 3.80 ± 0.22$^{ab}$ | 4.58 ± 0.22$^{a}$ | 3.73 ± 0.15$^{c}$ |
| II | 4.35 ± 0.09 | 4.15 ± 0.15$^{a}$ | 4.10 ± 0.23$^{ab}$ | 4.55 ± 0.06$^{a}$ |
| III | 4.26 ± 0.11 | 3.43 ± 0.27$^{c}$ | 3.09 ± 0.30$^{c}$ | 3.44 ± 0.21$^{c}$ |
| IV | 3.96 ± 0.13 | 3.38 ± 0.13$^{bc}$ | 3.86 ± 0.19$^{ab}$ | 3.84 ± 0.08$^{bc}$ |
| V | 4.08 ± 0.12 | 3.73 ± 0.08$^{abc}$ | 3.48 ± 0.15$^{bc}$ | 4.21 ± 0.15$^{ab}$ |
| VI | 4.09 ± 0.14 | 3.90 ± 0.18$^{ab}$ | 4.31 ± 0.23$^{a}$ | 4.76 ± 0.20$^{a}$ |

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

**Table 9. Effect of ethanolic and aqueous extracts of *Mangifera indica* on serum albumin levels, g/dl**

| Group | 0th day (Mean ± SE) | 9th day (Mean ± SE) | 15th day (Mean ± SE) | 30th day (Mean ± SE) |
|---|---|---|---|---|
| I | 3.96 ± 0.29 | 3.80 ± 0.22$^{b}$ | 4.58 ± 0.22$^{a}$ | 3.73 ± 0.15$^{b}$ |
| II | 4.35 ± 0.09 | 4.15 ± 0.15$^{b}$ | 4.10 ± 0.23$^{ab}$ | 4.55 ± 0.06$^{a}$ |
| VII | 4.07 ± 0.15 | 6.49 ± 0.32$^{a}$ | 4.04 ± 0.20$^{ab}$ | 4.63 ± 0.10$^{a}$ |
| VIII | 4.06 ± 0.16 | 3.75 ± 0.17$^{b}$ | 3.51 ± 0.23$^{b}$ | 3.84 ± 0.20$^{b}$ |
| IX | 4.00 ± 0.14 | 3.55 ± 0.09$^{b}$ | 3.80 ± 0.14$^{b}$ | 4.41 ± 0.23$^{a}$ |
| X | 4.23 ± 0.19 | 6.56 ± 0.31$^{a}$ | 4.13 ± 0.19$^{ab}$ | 4.76 ± 0.23$^{a}$ |

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

significantly (P>0.05). The mean values of Group VII (6.49 ± 0.32 g/dl) and Group X (6.56 ± 0.31 g/dl) also did not differ significantly. On 15$^{th}$ day, the mean albumin value of Group I was 4.58 ± 0.22 g/dl. Groups VIII and IX did not differ significantly (3.51 ± 0.23 and 3.80 ± 0.14 g/dl respectively). Groups II, VII and X showed intermediate values of 4.10 ± 0.23, 4.04 ± 0.20 and 4.13 ± 0.19 g/dl respectively. On 30$^{th}$ day, the albumin values of Groups II, VII, IX and X were comparable with mean values of 4.55 ± 0.06, 4.63 ± 0.10, 4.41 ± 0.23 and 4.76 ± 0.23 g/dl respectively. Group I (3.73 ± 0.15 g/dl) and Group VIII (3.84 ± 0.20 g/dl) did not differ significantly (P>0.05). The results indicated that the serum albumin levels were within the normal range in all the groups throughout the experiment.

#### *4.2.2.4. Total protein*

The results of the effect of ethanolic and aqueous extracts of *H. spinosa* on serum total protein levels are presented in Table 10. On 0$^{th}$ day, the mean serum total protein values were 5.50 ± 0.34, 6.73 ± 0.20, 6.43 ± 0.21, 4.18 ± 0.10, 6.13 ± 0.17 and 4.10 ± 0.41 respectively for Groups I to VI. On 9$^{th}$ day, the mean serum total protein levels of Group I and Group II were 5.52 ± 0.30 and 5.51 ± 0.14 g/dl respectively which did not differ significantly (P>0.05). The mean serum total protein levels in Group III (4.62 ± 0.22 g/dl) and Group IV (4.36 ± 0.25 g/dl) were comparable. Group V (6.82 ± 0.17 g/dl) and Group VI (6.49 ± 0.10 g/dl) also did not differ significantly (P>0.05). On 15$^{th}$ day, Group I and Group VI with mean serum total protein levels of 4.42 ± 0.13 g/dl and 4.70 ± 0.28 g/dl were comparable (P>0.05). The mean serum total protein levels of Groups II, III, IV and V (5.35 ± 0.18, 4.94 ± 0.36, 5.09 ± 0.06 and 5.97 ± 0.22 g/dl respectively) did not differ significantly (P>0.05). On 30$^{th}$ day, the mean serum total protein levels of Groups I to VI were 5.24 ± 0.33, 5.75 ± 0.03, 5.32 ± 0.35, 4.96 ± 0.32, 5.76 ± 0.13 and 4.53 ± 0.32 which did not differ significantly (P>0.05). The results indicated that the serum total protein levels were within the normal range in all the groups.

**Table 10. Effect of ethanolic and aqueous extracts of *Hygrophila spinosa* on serum total protein levels, g/dl**

| Group | 0th day (Mean ± SE) | 9th day (Mean ± SE) | 15th day (Mean ± SE) | 30th day (Mean ± SE) |
|---|---|---|---|---|
| I | 5.50 ± 0.34 | 5.52 ± 0.30$^{b}$ | 4.42 ± 0.13$^{b}$ | 5.24 ± 0.33$^{b}$ |
| II | 6.73 ± 0.20 | 5.51 ± 0.14$^{bc}$ | 5.35 ± 0.18$^{ab}$ | 5.75 ± 0.03$^{a}$ |
| III | 6.43 ± 0.21 | 4.62 ± 0.22$^{cd}$ | 4.94 ± 0.36$^{ab}$ | 5.32 ± 0.35$^{ab}$ |
| IV | 4.18 ± 0.10 | 4.36 ± 0.25$^{d}$ | 5.09 ± 0.06$^{ab}$ | 4.96 ± 0.32$^{a}$ |
| V | 6.13 ± 0.17 | 6.82 ± 0.17$^{a}$ | 5.97 ± 0.22$^{a}$ | 5.76 ± 0.13$^{a}$ |
| VI | 4.10 ± 0.41 | 6.49 ± 0.10$^{ab}$ | 4.70 ± 0.28$^{b}$ | 4.53 ± 0.32$^{ab}$ |

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

**Table 11. Effect of ethanolic and aqueous extracts of *Mangifera indica* on serum total protein levels, g/dl**

| Group | 0th day (Mean ± SE) | 9th day (Mean ± SE) | 15th day Mean ± SE) | 30th day (Mean ± SE) |
|---|---|---|---|---|
| I | 5.50 ± 0.34 | 5.52 ± 0.30$^{ab}$ | 4.42 ± 0.13$^{b}$ | 5.24 ± 0.33$^{ab}$ |
| II | 6.73 ± 0.20 | 5.51 ± 0.14$^{ab}$ | 5.35 ± 0.18$^{ab}$ | 5.75 ± 0.03$^{a}$ |
| VII | 6.12 ± 0.29 | 5.89 ± 0.19$^{a}$ | 5.44 ± 0.34$^{ab}$ | 5.34 ± 0.21$^{ab}$ |
| VIII | 6.10 ± 0.22 | 4.50 ± 0.30$^{b}$ | 5.98 ± 0.21$^{a}$ | 6.03 ± 0.16$^{a}$ |
| IX | 5.15 ± 0.21 | 4.98 ± 0.10$^{ab}$ | 5.13 ± 0.25$^{ab}$ | 4.47 ± 0.28$^{b}$ |
| X | 4.71 ± 0.18 | 4.68 ± 0.27$^{b}$ | 5.55 ± 0.24$^{ab}$ | 4.73 ± 0.17$^{b}$ |

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

The results of the effect of ethanolic and aqueous extracts of *M. indica* on serum total protein levels are shown in Table 11. On 0$^{th}$ day, the mean serum total protein levels of Groups VII to X were 6.12 ± 0.29, 6.10 ± 0.22, 5.15 ± 0.21 and 4.71 ± 0.18g/dl respectively. On 9$^{th}$ day, Groups I, II, VII, VIII, IX and X did not differ significantly with mean values of 5.52 ± 0.30, 5.51 ± 0.14, 5.89 ± 0.19, 4.50 ± 0.30, 4.98 ± 0.10 and 4.68 ± 0.27 g/dl respectively (P>0.05). On 15$^{th}$ day, Group I had mean serum total protein of 4.42 ± 0.13 g/dl while Group VIII had a mean serum total protein of 5.98 ± 0.21 g/dl. Groups II, VII and IX had intermediate levels of 5.35 ± 0.18, 5.44 ± 0.34 and 5.13 ± 0.25 g/dl which did not differ significantly (P>0.05). On 30$^{th}$ day, the mean serum total protein levels of Groups I, II, VII, VIII, IX and X were comparable which were 5.24 ± 0.33, 5.75 ± 0.03, 5.34 ± 0.21, 6.03 ± 0.16, 4.47 ± 0.28 and 4.73 ± 0.17 g/dl respectively. The results indicated that the serum total protein levels of all the groups were within the normal range throughout the experiment.

### *4.2.2.5. Serum sodium*

The results of the effect of ethanolic and aqueous extracts of *H. spinosa* on serum Na levels are presented in Table 12. On 0$^{th}$ day, the mean serum Na levels for Groups I to VI were 153.19 ± 2.81, 172.58 ± 2.01, 166.62 ± 2.43, 163.00 ± 3.20, 152.53 ± 3.07 and 159.97 ± 2.91 mEq/L respectively. On 9$^{th}$ day, the mean serum Na levels of Group I and Group II were comparable (170.86 ± 6.89 and 170.52 ± 3.13 mEq/L respectively) (P>0.05). The mean serum Na levels of Groups III, IV, V, VI were comparable (196.90 ± 2.58, 215.26 ± 2.76, 210.87 ± 2.24 and 214.78 ± 1.94 mEq/L respectively) (P>0.05). On 15$^{th}$ day, the mean serum Na levels of Groups I, III, IV and VI showed no significance difference (P>0.05) with mean values of 162.01 ± 1.66, 164.05 ± 1.77, 146.82 ± 2.81 and 162.25 ± 2.60 mEq/L respectively while Groups II and V were comparable (P>0.05) with mean values of 192.14 ± 2.59 and 181.67 ± 2.76 mEq/L respectively. On 30$^{th}$ day, the serum Na levels of all the groups were comparable (P>0.05) with mean values of 148.94 ± 1.54, 166.78 ± 5.52,

**Table 12. Effect of ethanolic and aqueous extracts of *Hygrophila spinosa* on serum Na levels, mEq/L**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 153.19 ± 2.81 | $170.86 \pm 6.89^{b}$ | $162.01 \pm 1.66^{b}$ | $148.94 \pm 1.54^{b}$ |
| II | 172.58 ± 2.01 | $170.52 \pm 3.13^{b}$ | $192.14 \pm 2.59^{a}$ | $166.78 \pm 5.52^{b}$ |
| III | 166.62 ± 2.43 | $196.90 \pm 2.58^{a}$ | $164.05 \pm 1.77^{b}$ | $165.05 \pm 2.43^{b}$ |
| IV | 163.00 ± 3.20 | $215.26 \pm 2.76^{a}$ | $146.82 \pm 2.81^{c}$ | $176.34 \pm 3.41^{b}$ |
| V | 152.53 ± 3.07 | $210.87 \pm 2.24^{a}$ | $181.67 \pm 2.76^{a}$ | $155.04 \pm 3.91^{b}$ |
| VI | 159.97 ± 2.91 | $214.78 \pm 1.94^{a}$ | $162.25 \pm 2.60^{bc}$ | $165.83 \pm 2.94^{b}$ |

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

**Table 13. Effect of ethanolic and aqueous extracts of *Mangifera indica* on serum Na levels, mEq/L**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 153.19 ± 2.81 | $170.86 \pm 6.89^{b}$ | $162.01 \pm 1.66^{b}$ | $148.94 \pm 1.54^{b}$ |
| II | 172.58 ± 2.01 | $170.52 \pm 3.13^{b}$ | $192.14 \pm 2.59^{a}$ | $166.78 \pm 5.52^{ab}$ |
| VII | 157.03 ± 2.72 | $215.11 \pm 3.04^{a}$ | $151.36 \pm 3.92^{b}$ | $201.94 \pm 2.70^{a}$ |
| VIII | 148.10 ± 2.52 | $167.32 \pm 3.60^{b}$ | $124.36 \pm 3.30^{b}$ | $181.04 \pm 3.11^{a}$ |
| IX | 162.61 ± 2.51 | $176.56 \pm 3.52^{b}$ | $151.00 \pm 3.10^{b}$ | $166.19 \pm 5.87^{ab}$ |
| X | 104.78 ± 2.44 | $130.03 \pm 2.12^{b}$ | $124.78 \pm 3.30^{b}$ | $169.71 \pm 2.29^{ab}$ |

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

165.05 ± 2.43, 176.34 ± 3.41, 155.04 ± 3.91 and 165.83 ± 2.94 mEq/L respectively for Groups I to VI. The results indicated that the serum Na levels of all the groups were within the normal range.

The results of the effect of ethanolic and aqueous extracts of *M. indica* on serum Na levels are presented in Table 13. On $0^{th}$ day, the mean serum Na levels of Groups VII, VIII, IX and X were 157.03 ± 2.72, 148.10 ± 2.52, 162.61 ± 2.51 and 104.78 ± 2.44 mEq/L respectively. On $9^{th}$ day, the mean serum Na level of Group VII was 215.11 ± 3.04 mEq/L while that of Group II was 170.52 ± 3.13 mEq/L. All the other treatment groups did not vary significantly (P>0.05) when compared with Group II with mean values of 167.32 ± 3.60, 176.56 ± 3.52 and 130.03 ± 2.12 mEq/L respectively for Groups VIII, IX and X. On $15^{th}$ day, the mean serum Na level of Group II was 192.14 ± 2.59 mEq/L. Groups I, VII, VIII, IX and X showed no significant difference (P>0.05) with mean values of 162.01 ± 1.66, 151.36 ± 3.92, 124.36 ± 3.30, 151.00 ± 3.10 and 124.78 ± 3.30 mEq/L respectively. On $30^{th}$ day, the mean serum Na levels of Group VII (201.94 ± 2.70 mEq/L) and Group VIII (181.04 ± 3.11 mEq/L) were comparable (P>0.05). The mean level of Group I was 148.94 ± 1.54 mEq/L. Groups II, IX and X had intermediate values with mean values of 166.78 ± 5.52, 166.19 ± 5.87, 169.71 ± 2.29 mEq/L respectively (P>0.05). The results indicated that the serum Na levels of all the groups were within normal range throughout the experiment.

#### *4.2.2.6. Serum potassium*

The results of the effect of ethanolic and aqueous extracts of *H. spinosa* on serum K levels are presented in Table 14. On $0^{th}$ day, the mean serum K levels of Groups I to VI were 192.60 ± 1.28, 83.95 ± 0.29, 131.87 ± 1.95, 115.79 ± 1.01, 115.88 ± 1.39 and 126.97 ± 1.15 mEq/L respectively. On $9^{th}$ day, mean serum K levels of Groups I and II were comparable with mean values of 149.16 ± 2.43 and 150.32 ± 2.56 mEq/L respectively (P>0.05). All the treatment groups (III, IV, V and

**Table 14. Effect of ethanolic and aqueous extracts of *Hygrophila spinosa* on serum K levels, mEq/L**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 192.60 ± 1.28 | $149.16 \pm 2.43^{b}$ | $106.87 \pm 0.64^{b}$ | $80.13 \pm 0.34^{c}$ |
| II | 83.95 ± 0.29 | $150.32 \pm 2.56^{b}$ | $93.04 \pm 0.69^{c}$ | $84.14 \pm 0.32^{c}$ |
| III | 131.87 ± 1.95 | $187.31 \pm 2.91^{a}$ | $149.43 \pm 2.76^{a}$ | $103.92 \pm 0.86^{b}$ |
| IV | 115.79 ± 1.01 | $179.83 \pm 0.96^{a}$ | $153.11 \pm 1.29^{a}$ | $120.36 \pm 0.11^{a}$ |
| V | 115.88 ± 1.39 | $193.71 \pm 1.17^{a}$ | $147.16 \pm 2.32^{a}$ | $112.76 \pm 1.20^{ab}$ |
| VI | 126.97 ± 1.15 | $188.51 \pm 2.42^{a}$ | $158.20 \pm 0.64^{a}$ | $119.46 \pm 0.73^{a}$ |

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

**Table 15. Effect of ethanolic and aqueous extracts of *Mangifera indica* on serum K levels, mEq/L**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 192.60 ± 1.28 | $149.16 \pm 2.43^{a}$ | $106.87 \pm 0.64^{a}$ | $80.13 \pm 0.34^{b}$ |
| II | 83.95 ± 0.29 | $150.32 \pm 2.56^{a}$ | $93.04 \pm 0.69^{c}$ | $84.14 \pm 0.32^{a}$ |
| VII | 103.76 ± 1.14 | $166.25 \pm 2.60^{a}$ | $108.21 \pm 1.06^{ab}$ | $84.90 \pm 0.45^{a}$ |
| VIII | 101.76 ± 2.27 | $157.61 \pm 2.63^{a}$ | $106.40 \pm 1.92^{ab}$ | $82.68 \pm 0.79^{ab}$ |
| IX | 99.68 ± 1.64 | $145.87 \pm 2.84^{a}$ | $108.50 \pm 0.84^{ab}$ | $88.43 \pm 0.24^{a}$ |
| X | 99.44 ± 2.67 | $169.54 \pm 1.82^{a}$ | $102.44 \pm 0.90^{bc}$ | $85.15 \pm 0.67^{a}$ |

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

VI) showed no significant variation in mean values (187.31 ± 2.91, 179.83 ± 0.96, 193.71 ± 1.17 and 188.51 ± 2.42 mEq/L respectively). On 15$^{th}$ day, the mean serum K value of Group I was 106.87 ± 0.64 mEq/L while that of group II was 93.04 ± 0.69 mEq/L. The serum K values of Groups III, IV, V and VI were comparable (P>0.05) with mean values of 149.43 ± 2.76, 153.11 ± 1.29, 147.16 ± 2.32 and 158.20 ± 0.64 mEq/L respectively. On 30$^{th}$ day, mean serum K levels of Group I was 80.13 ± 0.34 mEq/L while that of Group II was 84.14 ± 0.32 mEq/L (P>0.05). The mean serum K levels of Groups III, IV, V and VI were 103.92 ± 0.86, 120.36 ± 0.11, 112.76 ± 1.20 and 119.46 ± 0.73 mEq/L respectively which did not differ significantly (P>0.05). The results indicated that the serum K values of all the groups were within the normal range.

The results of the effect of ethanolic and aqueous extracts of *M. indica* on serum K levels are presented in Table 15. On 0$^{th}$ day, the mean serum K levels of Groups VII, VIII, IX and X were 103.76 ± 1.14, 101.76 ± 2.27, 99.68 ± 1.64 and 99.44 ± 2.67 mEq/L respectively. On 9$^{th}$ day, no significant variation was shown by Groups I, II, VII, VIII, IX and X with mean serum K levels of 149.16 ± 2.43, 150.32 ± 2.56, 166.25 ± 2.60, 157.61 ± 2.63, 145.87 ± 2.84 and 169.54 ± 1.82 mEq/L respectively. On 15$^{th}$ day, the mean serum K levels of Group I and Group II were 106.87 ± 0.64 and 93.04 ± 0.69 mEq/L respectively. All the other treatment groups showed no significant variation (P>0.05) with mean values of 108.21 ± 1.06, 106.40 ± 1.92, 108.50 ± 0.84 and 102.44 ± 0.90 mEq/L respectively for Groups VII, VIII, IX and X. On 30$^{th}$ day, the mean serum K levels of all the groups were comparable (P>0.05) with mean values of 80.13 ± 0.34, 84.14 ± 0.32, 84.90 ± 0.45, 82.68 ± 0.79, 88.43 ± 0.24 and 85.15 ± 0.67 mEq/L respectively for Groups I, II, VII, VIII, IX and X. The results indicated that all the groups showed a normal range of serum K levels.

*4.2.2.7. Serum calcium*

The results of the effect of ethanolic and aqueous extracts of *H. spinosa* on serum Ca levels are shown in Table 16. On $0^{th}$ day, the mean serum Ca levels of Groups I to VI were 8.38 ± 0.27, 8.73 ± 0.46, 8.60 ± 0.37, 9.50 ± 0.39, 8.97 ± 0.24 and 8.47 ± 0.35 mg/dl respectively. On $9^{th}$ day, the mean serum Ca levels of Group I (8.77 ± 0.37 mg/dl) and Group II (9.22 ± 0.47 mg/dl) were almost comparable. Groups III, IV and V had mean values of 8.97 ± 0.38, 9.41 ± 0.20 and 8.47 ± 0.07 mg/dl respectively which were also almost comparable. Group VI had a mean value of 10.05 ± 0.31 mg/dl. On $15^{th}$ day, the mean serum Ca level of Group II was 9.47 ± 0.38 mg/dl. No significant difference was shown by Groups I, III, IV, V and VI with mean values of 7.20 ± 0.30, 7.55 ± 0.14, 7.86 ± 0.22, 7.59 ± 0.11 and 8.40 ± 0.18 mg/dl respectively. On $30^{th}$ day, Groups I, III and V showed no significant variation with mean values of 5.95 ± 0.40, 6.46 ± 0.25 and 5.81 ± 0.09 mg/dl. The mean values (9.62 ± 0.42 mg/dl and 9.91 ± 0.33 mg/dl) of Groups II and VI respectively showed no significant variation ($P>0.05$). The results indicated that all the treatment groups had their serum Ca levels within the normal range.

The results of the effect of ethanolic and aqueous extracts of *M. indica* on serum Ca levels are presented in Table 17. On $0^{th}$ day, the mean serum Ca levels of Groups VII, VIII, IX and X were 7.88 ± 0.45, 6.22 ± 0.25, 8.69 ± 0.58 and 7.53 ± 0.33 mg/dl respectively. On $9^{th}$ day, no significant difference ($P>0.05$) was shown by Groups I, II and IX with mean serum Ca levels of 8.77 ± 0.37, 9.22 ± 0.47 and 8.82 ± 0.69 mg/dl respectively. Groups VII, VIII and X also showed no significant variation ($P>0.05$) with mean levels of 6.56 ± 0.28, 6.95 ± 0.27, 6.35 ± 0.41 mg/dl respectively. On $15^{th}$ day, Group I had a mean value of 7.20 ± 0.30 mg/dl while Group II had a mean value of 9.47 ± 0.38 mg/dl. Groups IX and X had intermediate levels of 8.17 ± 0.20 and 8.93 ± 0.60 mg/dl respectively. The mean serum Ca levels of Groups VII and VIII were 6.06 ± 0.14 and 5.85 ± 0.17 mg/dl respectively which showed no significant difference ($P>0.05$). On $30^{th}$ day, Groups II and VII were

**Table 16. Effect of ethanolic and aqueous extracts of *Hygrophila spinosa* on serum Ca levels, mg/dl**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 8.38 ± 0.27 | $8.77 \pm 0.37^{b}$ | $7.20 \pm 0.30^{c}$ | $5.95 \pm 0.40^{c}$ |
| II | 8.73 ± 0.46 | $9.22 \pm 0.47^{ab}$ | $9.47 \pm 0.38^{a}$ | $9.62 \pm 0.42^{b}$ |
| III | 8.60 ± 0.37 | $8.97 \pm 0.38^{b}$ | $7.55 \pm 0.14^{c}$ | $6.46 \pm 0.25^{c}$ |
| IV | 9.50 ± 0.39 | $9.41 \pm 0.20^{ab}$ | $7.86 \pm 0.22^{bc}$ | $11.05 \pm 0.17^{a}$ |
| V | 8.97 ± 0.24 | $8.47 \pm 0.07^{b}$ | $7.59 \pm 0.11^{c}$ | $5.81 \pm 0.09^{c}$ |
| VI | 8.47 ± 0.35 | $10.05 \pm 0.31^{a}$ | $8.40 \pm 0.18^{b}$ | $9.91 \pm 0.33^{b}$ |

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

**Table 17. Effect of ethanolic and aqueous extracts of *Mangifera indica* on serum Ca levels, mg/dl**

| Group | $0^{th}$ day (Mean ± SE) | $9^{th}$ day (Mean ± SE) | $15^{th}$ day (Mean ± SE) | $30^{th}$ day (Mean ± SE) |
|---|---|---|---|---|
| I | 8.38 ± 0.27 | $8.77 \pm 0.37^{a}$ | $7.20 \pm 0.30^{c}$ | $5.95 \pm 0.40^{b}$ |
| II | 8.73 ± 0.46 | $9.22 \pm 0.47^{a}$ | $9.47 \pm 0.38^{a}$ | $9.62 \pm 0.42^{a}$ |
| VII | 7.88 ± 0.45 | $6.56 \pm 0.28^{b}$ | $6.06 \pm 0.14^{d}$ | $10.20 \pm 0.37^{a}$ |
| VIII | 6.22 ± 0.25 | $6.95 \pm 0.27^{b}$ | $5.85 \pm 0.17^{d}$ | $5.58 \pm 0.14^{bc}$ |
| IX | 8.69 ± 0.58 | $8.82 \pm 0.69^{a}$ | $8.17 \pm 0.20^{bc}$ | $5.49 \pm 0.21^{bc}$ |
| X | 7.53 ± 0.33 | $6.35 \pm 0.41^{b}$ | $8.93 \pm 0.60^{ab}$ | $5.91 \pm 0.27^{bc}$ |

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

comparable with mean values of 9.62 ± 0.42 and 10.20 ± 0.37 mg/dl respectively. No significant difference was shown in the serum Ca values of Groups I, VIII, IX and X with mean values of 5.95 ± 0.40, 5.58 ± 0.14, 5.49 ± 0.21 and 5.91 ± 0.27 mg/dl respectively. The results indicated that all the groups had their serum Ca levels within the normal range.

**4.2.3. Histopathological examination of kidney**

In normal group (Group I), the microscopic examination of the kidney revealed the usual histological parameters. The tubular structures were largely intact without the presence of any mononuclear infiltrates in the interstitium (Fig.15).

In gentamicin group (Group II), there were extensive proximal tubular necrosis and loss of the lining epithelium and these features were predominantly subcapsular. Besides, there were interstitial oedema, perivascular oedema and multiple focal collections of mononuclear cells in the interstitium. The glomerular changes were quite marked (Fig. 16).

In *H. spinosa* ethanolic 50 mg/kg treated group (Group III), there were areas of tubular degeneration and necrosis along with areas of perivascular oedema and tubulo-interstitial mononuclear cell infiltrates at different foci throughout the cortex (Fig. 17).

In *H. spinosa* ethanolic 250 mg/kg treated group (Group IV), the proximal tubular epithelial cells showed varying degrees of regeneration but slight degenerative changes. Besides this, there were scattered small foci of mononuclear cell infiltration confined to subcapsular area. The epithelial cells of the proximal convoluted tubules were more or less intact. However, a few cells showed mild degenerative changes characterized by vacuolar cytoplasm (Fig. 18).

In *H. spinosa* aqueous 50 mg/kg treated group (Group V), the renal tubular cells showed varying degrees of dilatation with hyaline cast formation in the lumen

and the lining tubular epithelial cells showed varying levels of vacuolar degeneration and necrosis (Fig. 19).

In *H. spinosa* aqueous 250 mg/kg treated group (Group VI), areas of tubular degeneration and necrosis were observed at a few foci in the cortex along with varying degrees of regenerative changes (Fig. 20).

In *M. indica* ethanolic 100 mg/kg treated group (Group VII), moderate areas of tubular vacuolar degeneration and necrosis with a few collections of mononuclear cells in the interstitium were observed (Fig. 21).

In *M. indica* ethanolic 500 mg/kg treated group (Group VIII), the section revealed a few scattered areas of mild tubular degeneration and necrosis. At places, the distal convoluted tubules (DCT) were dilated with hyaline casts (Fig. 22).

In *M. indica* aqueous 100 mg/kg treated group (Group IX), variable degrees of tubular degeneration, vascular congestion, perivascular oedema and mononuclear cell infiltration and aggregation in the tubulo-interstitial spaces were observed (Fig. 23).

In *M. indica* aqueous 500 mg/kg treated group (Group X), a few small foci of subcapsular tubular necrosis were observed and some of the tubules of DCT showed varying degrees of dilatation and hyaline cast formation in their lumen (Fig. 24).

The results of histopathological examination of the kidney revealed significant changes between ethanolic extracts of *H. spinosa* at doses 50 mg/kg and 250 mg/kg. Ethanolic extract of *M. indica* at doses 100 mg/kg and 500 mg/kg also produced significant histological changes. Ethanolic extract of *H. spinosa* at a dose rate of 250 mg/kg produced more regenerative changes in the kidney than the ethanolic extract of *M. indica* at a dose rate of 500 mg/kg.

Histologically, no significant renal changes could be observed between aqueous extracts of *H. spinosa* at doses 50 mg/kg and 250 mg/kg. Similar is the case with aqueous extracts of *M. indica* at doses 100 mg/kg and 500 mg/kg.

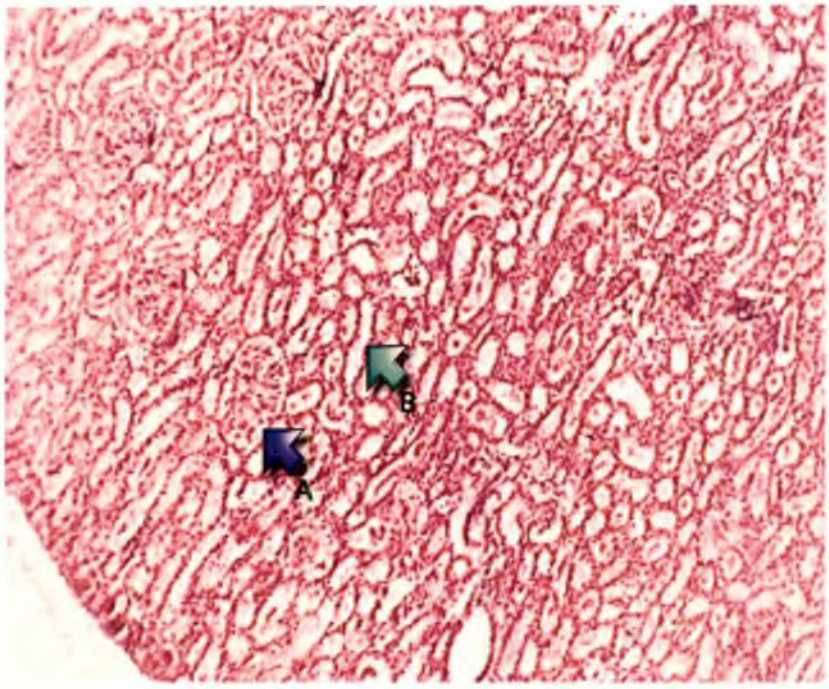


Fig. 15. Normal group (H & E x 100)
A : intact glomerulus
B : intact proximal convoluted tubules

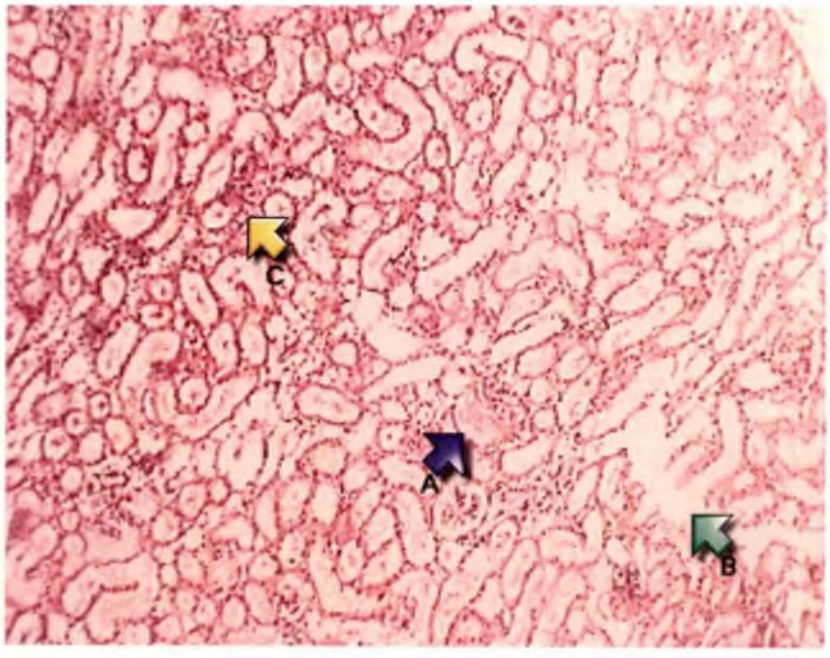


Fig. 16. Gentamicin group (H & E x 100)
A : marked glomerular changes
B : extensive tubular necrosis
C : mononuclear cell infiltration in the interstitium

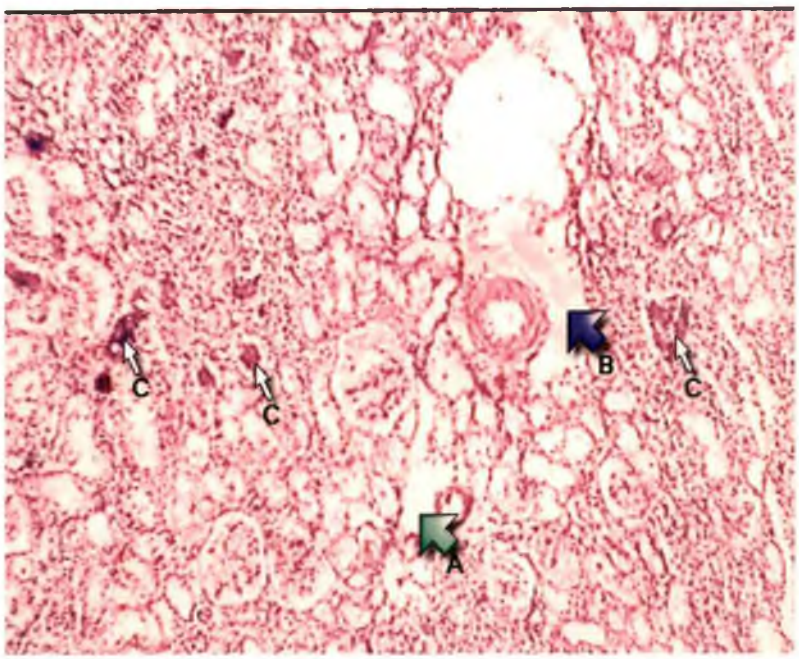


Fig. 17. Ethanolic *H. spinosa* ( 50 mg/kg) treated group
(H & E x 100)

A : tubular degenaration and necrosis
B : perivascular oedema
C : different foci of mononuclear infiltration

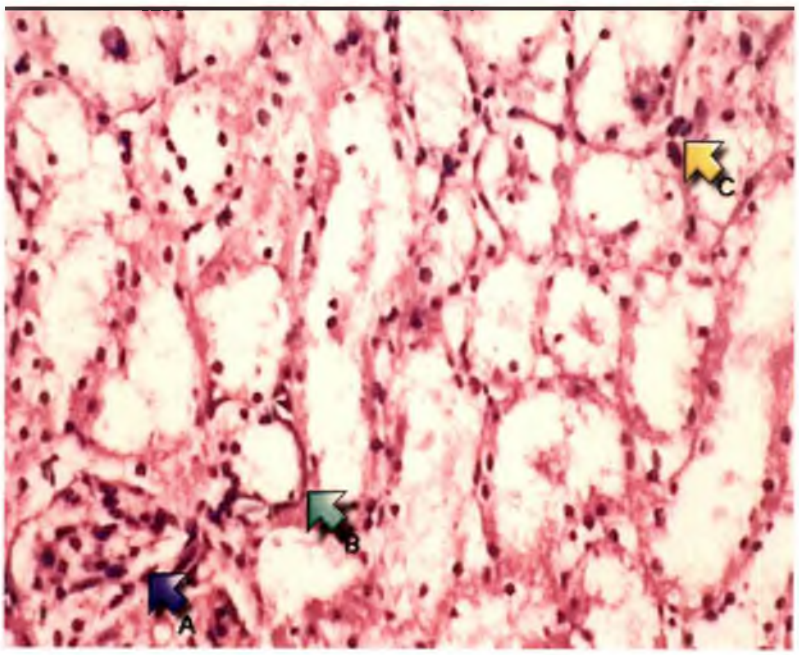


Fig. 18. Ethanolic *H. spinosa* (250 mg/kg) treated group
( H & E x 400)

A : minimal glomerular changes
B : regenerative proximal tubules
C : foci of mononuclear cell infiltration

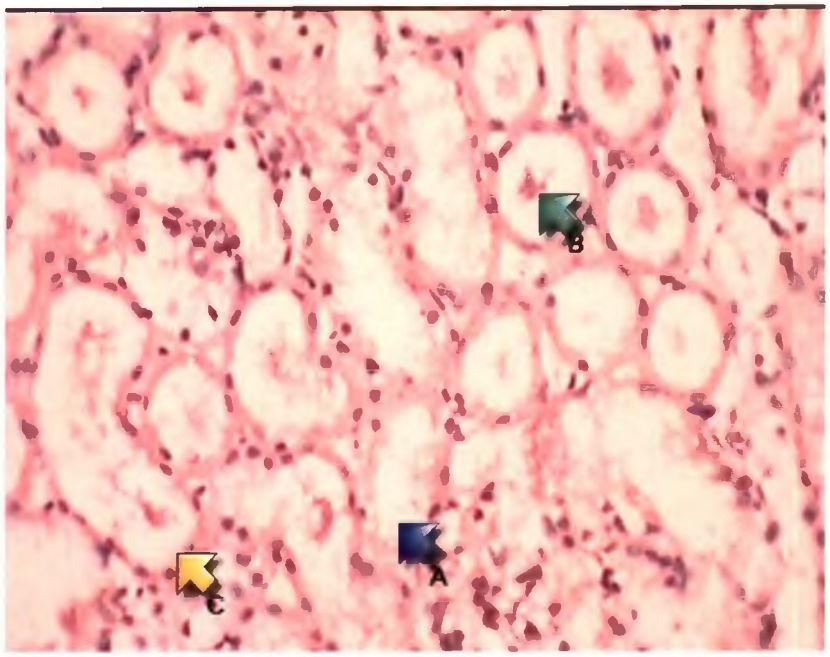


Fig. 19. Aqueous *H. spinosa* (50 mg/kg) treated group
(H & E x 400)

A : tubular degenarative changes
B : hyaline casts in distal convoluted tubules
C : dilated lumen of distal convoluted tubules

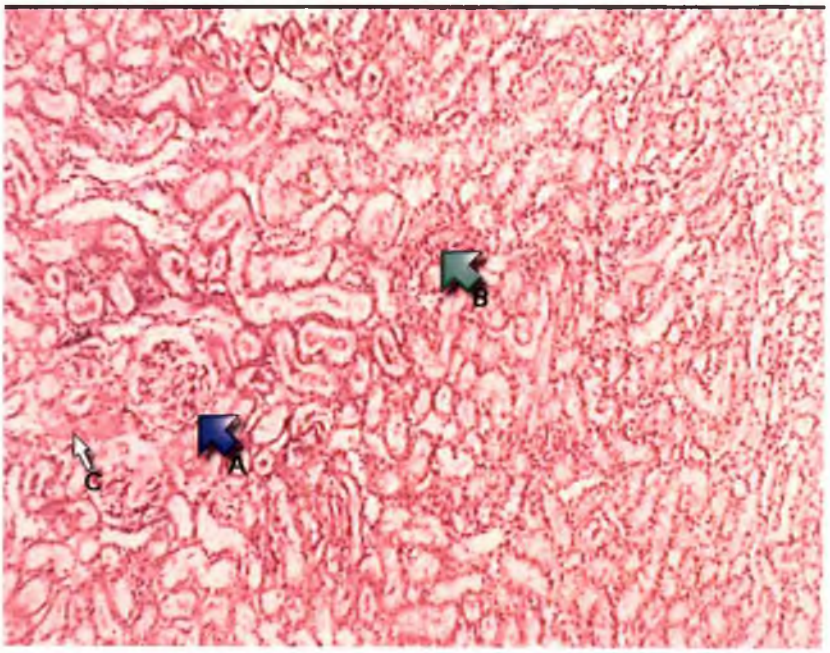


Fig. 20. Aqueous H. spinosa (250 mg/kg) treated group
(H & E x 100)

A : minimal glomerular changes
B : varrying degrees of tubular regenerative changes
C : subcapsular areas of degeneration

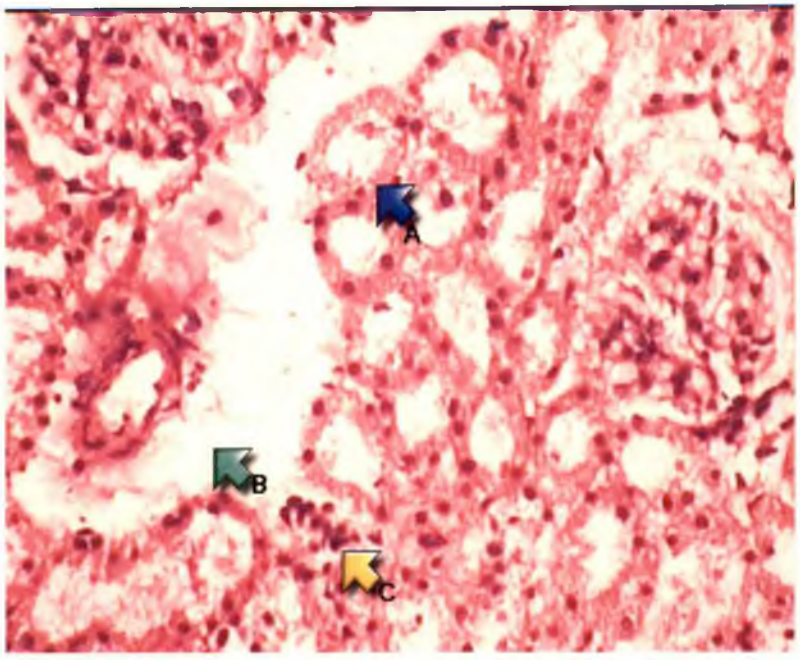


Fig. 21. Ethanolic *M. indica* (100 mg/kg) treated group
(H & E x 400)

A : vascular changes in the proximal convoluted tubules
B : perivascular oedema
C : focal collection of mononuclear cells in the interstitium

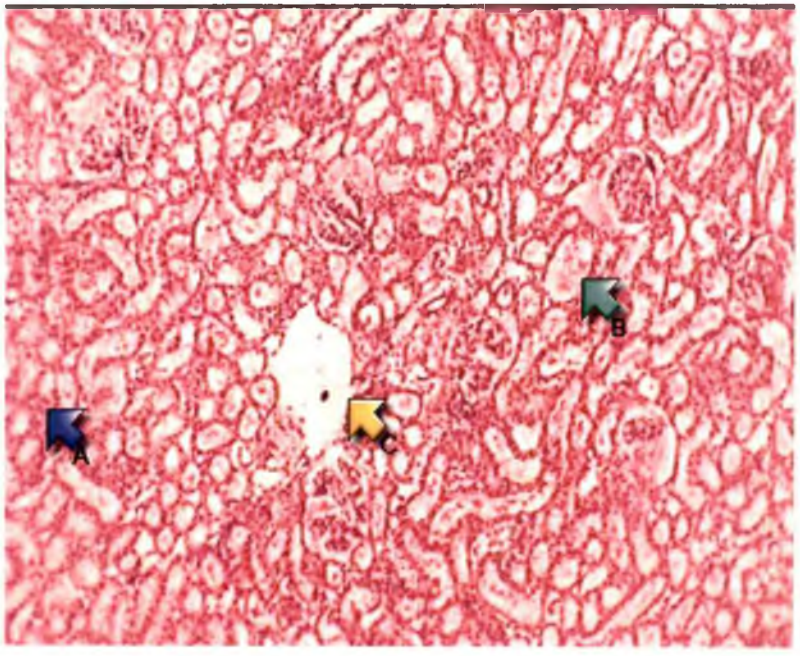


Fig. 22. Ethanolic *M. indica* (500 mg/kg) treated group
(H & E x 100)

A : mild tubular degenaration in subcapsular area
B : hyaline cast in distal convoluted tubule
C : dilated lumen of distal convoluted tubule

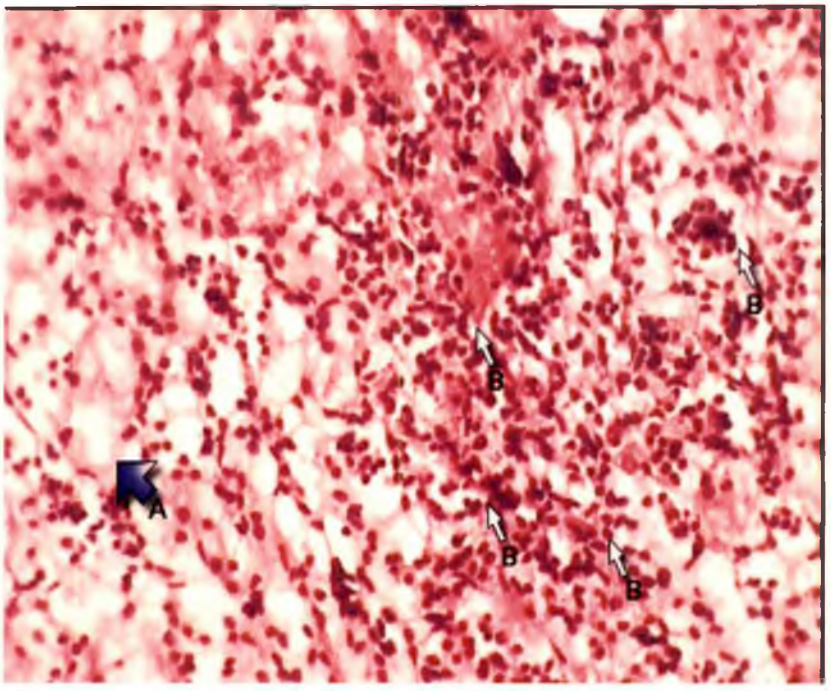


Fig. 23. Aqueous *M. indica* (100 mg/kg) treated group
(H & E x 400)
A : vacuolar changes in the proximal convoluted tubule
B : scattered mononuclear cell infiltration in the cortex

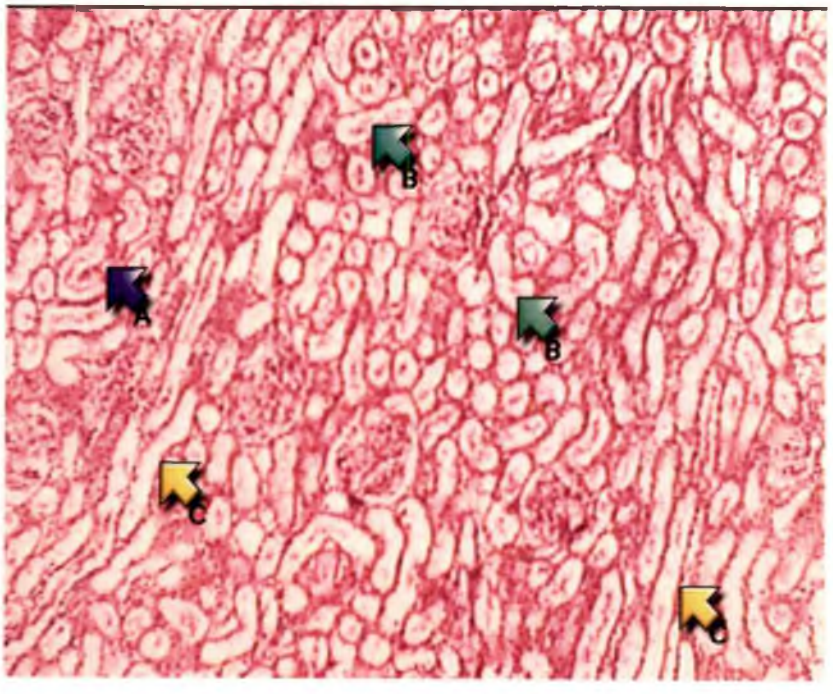


Fig. 24. Aqueous *M. indica* (500 mg/kg) treated group
(H & E x 100)
A : mild tubular degenaration and necrosis in the subcapsular area
B : multiple hyaline cast formation in distal convoluted tubule
C : pronounced dilatation of distal convoluted tubule

# *Discussion*

# 5. DISCUSSION

Nephrotoxicity occurs as a disturbance in renal function due to various adverse drug interactions, inadequate elimination of radioactive contrast materials and chemicals. It is of great concern in patients with renal failure. Nephrotoxicity may limit the clinical usefulness of many diagnostic and therapeutic agents; recognition of factors associated with higher risk for renal injury is of great importance. However, the end point of nephrotoxicity is always cell death; therefore, it is important to identify the mechanism in addition to the site of action, in order to formulate a strategy for damage prevention. The strategies aimed at ameliorating the nephrotoxicity are of clinical interest.

A standard drug which provides nephroprotection is still a major question yet to be answered. Many herbal drugs with action on the urinary system are widely used in human and veterinary medicine in nephrotoxic cases. These herbal drugs are used traditionally but their efficacy and beneficial effects are not scientifically documented. Hence the present study was undertaken to assess the nephroprotective effect of *Hygrophila spinosa* and *Mangifera indica* in gentamicin-induced nephrotoxicity.

## 5.1. PHYTOCHEMICAL SCREENING

Phytochemical screening of the ethanolic extract of *Hygrophila spinosa* revealed the presence of steroids, phenolic compounds, flavonoids, diterpenes and saponins while the aqueous extract of *Hygrophila spinosa* revealed the presence of phenolic compounds, tannins and saponins. Steroids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes and saponins were detected in the ethanolic extract of *Mangifera indica* while its aqueous extract yielded phenolic compounds, tannins, glycosides and diterpenes.

Misra *et al.* (2001) reported that *Asteracantha longifolia* Syn. *Hygrophila spinosa* contained flavonoids, terpenoids, sterols, betulin, 25-oxo-hentria contanyl acetate and methyl 8-n-hexyltetracosanoate. Hewawasam *et al.* (2003) stated that flavonoids were known to be antioxidant, free radical scavengers and antiperoxidants and these flavonoids in *Asteracantha longifolia* provided hepatoprotection against carbon tetrachloride and paracetamol-induced hepatotoxicity in mice. Flavonoids are reported to possess antioxidant activity by Catapano (1997).

According to Nadkarni (1976) the major constituent in the stem bark of *Mangifera indica* is tannin and stem bark of *Mangifera indica* is said to be astringent and used as a tonic. The studies conducted by Prashanth (2001) revealed the presence of tannins, sterols and xanthones in the ethanolic extract of *Mangifera indica* bark. The stem bark of *Mangifera indica* contained polyphenols, triterpenes, flavonids and phytosterols. In general, these compounds are said to be antioxidant (Garrido *et al.*, 2001). Sato *et al.* (1998) reported that the main polyphenol in the stem bark of *Mangifera indica* is a C-glucosylxanthone, mangiferin, exhibited antioxidant acitivity in *in vitro* systems. The scavenger ability of norathyriol (aglycone of mangiferin) was demonstrated in a model of respiratory burst induction in rat neutrophils (Hsu *et al.*, 1997).

The nephroprotective effect of plants under this study may be attributed to the presence of phytochemical constituents like flavonoids and phenolic compounds, mainly through their antioxidant property.

## 5.2. OBSERVATIONS

### 5.2.1. Antioxidant effect

In the present study, the ethanolic extracts as well as the aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* exhibited significant antioxidant effect in a dose-dependent manner.

*5.2.1.1. Superoxide dismutase*

In the present study, superoxide dismutase levels were decreased in gentamicin treated groups which correlates with the findings of Ali *et al.* (2005) and Kozat *et al.* (2007). Treatment with the ethanolic and aqueous plant extracts showed a significant increase in superoxide dismutase level. A slightly higher significant increase in superoxide dismutase level was noted in ethanolic extracts of *Hygrophila spinosa* and *Mangifera indica* at doses 250 mg/kg and 500mg/kg respectively.

Studies conducted by Vijaykumar *et al.* (2006) reported that ethanolic extracts of *Hygrophila auriculata* at doses 100 and 250 mg/kg produced an increase in the activity of superoxide dismutase, catalase, glutathione, glutathione peroxidase and decrease in lipid peroxidation suggesting the antioxidant property at higher dose rate.

Sanchez *et al.* (2000) found out that the decoction of stem bark of *Mangifera indica* caused an increase in superoxide dismutase level in 12-o-tetradecaecanoylphorbol-13-acetate (TPA)-induced oxidative damage in mice. Anila and Vijayalakshmi (2003) have reported that the ethyl acetate fraction of ground dried materials of *Mangifera indica* caused an increase in the levels of superoxide dismutase, catalase, reduced glutathione and a decrease in lipid peroxidation.

In the present study, the treatment with *Hygrophila spinosa* and *Mangifera indica* enhanced the superoxide dismutase levels which gives an indication of the antioxidant potential of these plant extracts.

The significance between the ethanolic extract of *Hygrophila spinosa* at 250 mg/kg and the ethanolic extract of *Mangifera indica* at 500 mg/kg was tested and found that the ethanolic extract of *Mangifera indica* at 500 mg/kg had higher superoxide dismutase level (P=0.00). Eventhough the ethanolic extract of *Mangifera indica* showed higher value, the ethanolic extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg produced the significant reduction in the enzymatic activity at the

lower dose itself. Thus it is concluded that the ethanolic extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg is more effective in providing nephroprotection by way of increasing superoxide dismutase levels than the ethanolic extract of *Mangifera indica* at a dose rate of 500 mg/kg.

### *5.2.1.2. Lipid peroxidation*

Lipid peroxidation, measured by the formation of thio barbituric acid reactive substances, was significantly increased in gentamicin treated groups. This is in agreement with earlier studies of Abdel-Naim *et al.* (1999) and Ali (2002). Reactive oxygen species have been proposed to cause cell death in many different pathological states in humans and animals. Reactive oxygen species produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage.

In the present study, the ethanolic and aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* at lower doses did not considerably reduce the increased lipid peroxidation level. But higher doses of the extracts showed considerable reduction in lipid peroxidation level. Maximum reduction was shown by ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg and aqueous extract of *Mangifera indica* at the dose rate of 500 mg/kg.

Shanmugasundaram and Venkataraman (2006) proved the *in vitro* antioxidant property of aqueous root extract of *Hygrophila auriculata* by thiobarbituric acid method. Vijayakumar *et al.* (2006) reported that ethanolic extract of *Hygrophila auriculata* caused a reduction in lipid peroxidation at doses of 100 mg/kg and 250 mg/kg.

Martinez *et al.* (2000) showed that a significant inhibitory effect on the peroxidation of rat brain phospholipids and powerful scavenging activity of hydroxyl radicals was shown by aqueous stem bark extract of *Mangifera indica.* Martinez *et al.* (2001) reported that QF808 formulation, prepared as decoction of stem bark of

*Mangifera indica*, was proved to be antioxidant due to its ability to scavenge free radicals involved in microsome lipid peroxidation.

In the present study also, the tissue lipid peroxidation level was reduced following treatment with the plant extracts which may favour their nephroprotective effect.

The significance between the ethanolic extract of *Hygrophila spinosa* at 250 mg/kg and the aqueous extract of *Mangifera indica* at 500 mg/kg was tested and found that the aqueous extract of *Mangifera indica* at 500 mg/kg showed maximum reduction in lipid peroxidation value (P=0.00). But the ethanolic extract of *Hygrophila spinosa* at 250 mg/kg produced the antioxidant effect with reduction in lipid peroxidation at the lower dose. Thus it is concluded that the ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg is more effective in reducing the lipid peroxidation than the aqueous extract of *Mangifera indica* at 500 mg/kg.

*5.2.1.3. Catalase*

Catalase activity was significantly reduced in gentamicin treated groups when compared with the control group. This result was supported by the findings of Pedraza-Chaverri *et al.* (2000) in which they found out that catalase activity was significantly decreased in gentamicin alone treated group while the garlic treatment was unable to prevent the decrease in catalase activity. All the plant extract treatment groups, in the present study, showed a significant increase in the catalase activity. Among them, the maximum increase in catalase activity was shown by ethanolic extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg and aqueous extract of *Mangifera indica* at a dose rate of 500 mg/kg.

Vijayakumar *et al.* (2006) reported that *Hygrophila auriculata* caused an increase in the activity of catalase in streptozotocin-induced oxidative damage in male Sprague-Dawley rats. The studies conducted by Shifow *et al.* (2000) found out that melatonin at the dose rate of 5 mg/kg prevented the gentamicin-induced fall in

the catalase level. *Spirulina fusiformis* was shown to enhance catalase activities in gentamicin-induced oxidative stress and renal dysfunction (Kuhad *et al.*, 2006).

Similar results are obtained in the present study with the ethanolic and aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* suggesting their efficacy in preventing free radical-induced damage thereby contributing to the nephroprotective action of the plant extracts.

The significance between the ethanolic extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg and the aqueous extract of *Mangifera indica* at a dose rate of 500 mg/kg was tested and the results indicated that the aqueous extract of *Mangifera indica* at 500 mg/kg produced maximum increase in catalase value (P=0.00). Since the ethanolic extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg produced an increase in the catalase activity at the lower dose itself, it is interpreted that the ethanolic extract of *Hygrophila spinosa* at 250 mg/kg is more effective in increasing the catalase activity than the other treatment.

#### *5.2.1.4. Reduced glutathione*

Gentamicin treatment caused a significant decrease in reduced glutathione level which agrees with the findings of Kozat *et al.* (2007) in which they reported that gentamicin decreased the levels of reduced glutathione. The treatment with the ethanolic and aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* showed considerable increase in the reduced glutathione levels. The maximum reduced glutathione levels were observed in the ethanolic extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg and ethanolic extract of *Mangifera indica* at 500 mg/kg. Grape seed extract was found to increase the reduced glutathione level in gentamicin-induced nephrotoxicity (El-Ashmawy *et al.*, 2006). The studies conducted by Ali and Mousa (2001) revealed that reduced glutathione level was increased by dimethyl sulfoxide in gentamicin-induced nephrotoxicity. Similar results were shown by Ebselen, a selenoorganic drug, in gentamicin-induced renal

damage (Dhanarajan *et al.*, 2006). Increase in reduced glutathione level of ethanolic and aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* which may enhance the antioxidant potential of these plant extracts thus contributing to their nephroprotective effect.

The significance tested between the ethanolic extracts of *Hygrophila spinosa* and *Mangifera indica* at doses 250 mg/kg and 500 mg/kg respectively indicated that the ethanolic extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg showed maximum reduction in the enzymatic activity (P=0.00) (by comparing the mean values) demonstrating the more effective nature of the treatment.

In the present study, the ethanolic and aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* at various doses produced significant decrease in the lipid peroxidation and increase in superoxide dismutase, catalase and reduced glutathione levels suggesting the strong antioxidant effect of these extracts. Ethanolic and aqueous extracts of *Hygrophila spinosa* at a dose rate of 250 mg/kg caused an increase in superoxide dismutase and catalase levels and a decrease in lipid peroxidation. Surprisingly, aqueous extract of *Hygrophila spinosa* at a dose rate of 50 mg/kg produced an increased reduced glutathione level. Among *Mangifera indica* treated groups, ethanolic and aqueous extracts of *Mangifera indica* at a dose rate of 500 mg/kg produced increased superoxide dismutase, catalase and reduced glutathione levels and decreased lipid peroxidation in gentamicin-induced nephrotoxicity. The significance tested between *Hygrophila spinosa* and *Mangifera indica* treated groups revealed that ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg is more effective than the other treatment groups.

Many investigators have described the role of reactive oxygen species (ROS) including hydroxyl radicals in gentamicin-induced nephrotoxicity (Baliga *et al.*, 1997, Walker *et al.*, 1999, Al-Majed *et al.*, 2002). The results of the present experiment confirms the above findings that gentamicin-induced nephrotoxicity

caused an increase in lipid peroxidation in the renal tissue as reflected by increase in malondialdehyde (MDA) and a decrease in superoxide dismutase, catalase and reduced glutathione levels. The nephrotoxicity produced by gentamicin was reversed by the plant extracts under study, indicating the antioxidant effect of these plant extracts. The antioxidant effect of the plant extracts may be attributed to the inhibition of oxidative damage caused by gentamicin, due to the presence of phytoconstituents. Kozat *et al.* (2007) came to a conclusion that administration of antioxidant substances in gentamicin-induced nephrotoxicity could ameliorate the toxicity.

### 5.2.2. Serum parameters

Serum creatinine and serum urea (serum markers of kidney function) have been considered the most important manifestations of severe tubular necrosis of kidney (Ali *et al.*, 2001, Afzal *et al.*, 2004).

#### *5.2.2.1. Effect on creatinine*

Serum creatinine levels were significantly increased in gentamicin group. These results are in accordance with the findings of Ramsammy *et al.* (1989) that the serum creatinine concentration was elevated significantly in gentamicin nephrotoxicity. Elevation of serum creatinine was marked on $15^{th}$ and $30^{th}$ day of the experiment in gentamicin group. The ethanolic and aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* at higher doses showed a significant reduction in serum creatinine level on $15^{th}$ day while their lower doses showed a gradual decrease in serum creatinine (significant reduction showed on the $30^{th}$ day) and by the end of experiment, the serum creatinine levels of all the treatment groups were comparable with that of normal group. Ethanolic extracts of *Hygrophila spinosa* and *Mangifera indica* at doses 250 mg/kg and 500 mg/kg respectively showed maximum reduction in serum creatinine level.

The studies conducted by Shirwaikar *et al.* (2004) proved that *Aerva lanata* provided nephroprotection indicated by reduction in serum creatinine level which was elevated by gentamicin administration. Kotnis *et al.* (2004) reported that *Hemidesmus indicus*, a herbal drug, ameliorated the increased serum creatinine levels thereby providing nephroprotection. According to Pedraza-Chaverri *et al.* (2003), diallyl sulfide, a garlic derived compound with antioxidant properties, decreased the elevated serum creatinine in gentamicin-induced nephrotoxicity. Ali (2002) observed that the serum index of nephrotoxicity, namely serum creatinine, elevated following gentamicin injection, was ameliorated by administration of *Rhazya stricta* leaves.

Similar results are also shown by the plant extracts under study where these herbal extracts caused a reduction in the creatinine values thereby providing a striking nephroprotective effect.

The significance tested between the ethanolic extracts of *Hygrophila spinosa* and *Mangifera indica* treated groups at 250 mg/kg and 500 mg/kg respectively revealed that both the treatment groups were equally effective (P=0.08). But, the ethanolic extract of *Hygrophila spinosa* produced the significant reduction in serum creatinine value at the lower dose. Thus it is concluded that the ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg is more effective in providing nephroprotection.

***5.2.2.2. Effect on urea***

Gentamicin administration caused an elevation in the level of serum urea. This is in accordance with the findings of Kozat *et al.* (2007) where elevated serum urea levels were found in gentamicin group. By the end of the experiment, the serum urea levels were significantly reduced in the treatment groups when compared with the control group. Maximum reduction in serum urea levels were shown by ethanolic

extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg and aqueous extract of *Mangifera indica* at a dose rate of 500 mg/kg.

Ali *et al.* (2005) observed that elevated serum urea level was decreased by curcumin at a dose rate of 200 mg/kg in gentamicin-induced renal damage. According to El-Ashmawy *et al.* (2006), grape seed extract prevented gentamicin-induced nephrotoxicity in mice by decreasing the serum urea and serum creatinine levels. *Jawarish Zarooni Sada*, a known nephroprotective preparation, was found to decrease serum urea and serum creatinine levels in gentamicin-induced nephrotoxicity in rats (Afzal *et al.*, 2004). Ali (2002) observed that the leaves of the medicinal plant *Rhazya stricta* produced a significant reduction in the serum urea levels within 10 days of treatment in male Wistar rats following gentamicin administration.

The results of the present study are also in accordance with the above findings and the plant extracts showed significant reduction in serum urea levels thereby contributing towards the nephroprotective effect.

The significance between the ethanolic extract of *Hygrophila spinosa* at 250 mg/kg and the aqueous extract of *Mangifera indica* at 500 mg/kg was tested and found that the ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg is more effective in reducing the serum urea level than the aqueous extract of *Mangifera indica* at 500 mg/kg (P=0.00).

***5.2.2.3. Effect on albumin, total protein, sodium, potassium and calcium***

The results of serum parameters like albumin, total protein, sodium, potassium and calcium revealed no significant variation. All these parameters were within the normal range (Hrapkiewicz *et al.*, 1998).

The study conducted by Kotnis *et al.* (2004) to assess the nephroprotective effect of *Hemidesmus indicus* in gentamicin-induced renal toxicity in rats revealed no difference in the levels of serum albumin, total protein, sodium and potassium as

compared with the gentamicin and normal control groups. Humes *et al.* (1984) pointed that the serum calcium level showed no significant variation in gentamicin-induced nephrotoxicity.

These findings are in accordance with the present study that no significant variation was observed in serum albumin, total protein, sodium, potassium and calcium, and gentamicin-induced nephrotoxicity had no influence on these parameters.

**5.2.3. Histopathological examination of kidney**

Histologically, gentamicin group showed severe proximal tubular necrosis and loss of lining epithelium. Besides, there were mononuclear cell infiltrations. In the ethanolic extract of *Hygrophila spinosa* 250 mg/kg treated group, varying degrees of regeneration and only small foci of mononuclear infiltration could be seen. In the ethanolic extract of *Hygrophila spinosa* 50 mg/kg treated group, there were areas of degeneration and mononuclear cell infiltration at different foci. In the ethanolic extract of *Mangifera indica* 500 mg/kg treated group, mild tubular degeneration and necrosis could be observed whereas in the ethanolic extract of *Mangifera indica* 100 mg/kg treated group, tubular vacuolar degeneration and necrosis with mononuclear infiltration could be seen. No significant changes could be observed between the aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* at various doses.

Karatas *et al.* (2004) studied the effect of tempol on gentamicin-induced nephrotoxicity and histopathological examination of the specimens revealed tubular regeneration and mononuclear cell infiltration in gentamicin plus tempol groups. Studies conducted by Vardi *et al.* (2005) found that gentamicin administration showed marked tubular necrosis and desquamation of the cortical epithelial cells and these changes were ameliorated by caffeic acid phenethyl ester. The base membrane alterations were less severe in caffeic acid phenethyl ester treated group. El-

Ashmawy *et al.* (2006) found that gentamicin group showed hydropic degenerated tubular epithelium, congested blood vessels, perivascular oedema, lymphocytic infiltrations and aggregations, renal tubules with varying degrees of dilatation and hyaline casts formation, and pretreatment with grape seed extract prevented the gentamicin-induced renal damage.

Ali and Mousa (2001) observed that gentamicin group showed moderate degree of renal tubular necrosis while dimethyl sulphoxide treated groups had a mild degree of tubular necrosis. Dhanarajan *et al.* (2006) observed that histologically, pretreatment with Ebselen ameliorated gentamicin-induced renal lesions. Moderate to severe tubular necrosis of the proximal and distal convoluted tubules and necrosis of the glomerulus as well as collecting ducts were observed in the gentamicin group. Pretreatment with Ebselen showed mild tubular necrosis and normal medullary architecture. In the present study also, these histological changes were observed.

Ethanolic extract of *Hygrophila spinosa* at a dose rate of 250 mg/kg showed varying degrees of regenerative stages than that of the other treatment groups. The epithelial cells of the proximal convoluted tubules of this group were intact. In this group, the glomerular changes were scanty. These histopathological findings supported by the results of antioxidant and serum biochemical parameters demonstrated the more nephroprotective action of ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg than the other treatment groups.

Gentamicin nephrotoxicity occurs as a result of binding of the cationic aminoglycoside with anionic phosphatidyl inositol which are present in the kidney. Recently, it is proposed that aminoglycoside nephrotoxicity results from endocytic retrieval of the drug by megalin, an endocytic-mediated receptor, present in the apical membrane of proximal convoluted tubules. However, the generation of reactive oxygen species (ROS) has been one of the major contributing factors towards nephrotoxicity. The mechanism of action through which these plant extracts

provide nephroprotection is not clearly understood, but may be due to the scavenging of the free radicals that were generated by gentamicin administration mediated by the action of phytoconstituents present in the plant extracts. The more nephroprotective effect of the ethanolic extract of *Hygrophila spinosa* may be attributed to the excretion of toxic substances along with increased diuresis due to high potassium content of the plant. Further investigations are required to elucidate the exact mechanism(s) underlying this possible beneficial effect.

Thus, the findings of the present study validate the nephroprotective effect of *Hygrophila spinosa* and *Mangifera indica* for the management of renal disorders.

# *Summary*

## 6. SUMMARY

The present study was undertaken to assess the nephroprotective effect of whole plant of *Hygrophila spinosa* and stem bark of *Mangifera indica* against gentamicin-induced nephrotoxicity in albino rats.

Eighty adult male Sprague Dawley rats weighing 200-250g, divided into ten groups comprising eight animals in each group, were used for the study. The experiment was conducted for a period of 30 days. Group I served as healthy control which was administered with 0.125 percent Tween 80 (vehicle) for 30 days. Gentamicin sulphate was administered to Groups II, III, IV, V, VI, VII, VIII, IX and X at a dose rate of 80 mg/kg i.p. for eight days. Group II was retained as such without any treatment till the completion of the experiment. Groups III and IV were administered with ethanolic extract of *Hygrophila spinosa* at a dose rate of 50 mg/kg and 250 mg/kg respectively for 22 days following gentamicin administration. Groups V and VI were administered with aqueous extract of *Hygrophila spinosa* at a dose rate of 50 mg/kg and 250 mg/kg respectively for 22 days following gentamicin administration. Ethanolic extract of *Mangifera indica* was administered at doses 100 mg/kg and 500 mg/kg to Groups VII and VIII respectively from $9^{th}$ day to $30^{th}$ day (22 days) of the experiment following gentamicin administration for eight days. Similarly, aqueous extract of *Mangifera indica* was administered at doses 100 mg/kg and 500 mg/kg respectively from $9^{th}$ day to $30^{th}$ day following gentamicin administration for eight days.

The blood was collected from all the animals on $0^{th}$, $9^{th}$, $15^{th}$ and $30^{th}$ day and serum was used for the estimation of creatinine, urea, albumin, total protein, sodium, potassium and calcium. On $30^{th}$ day, all the animals were sacrificed and kidney was used for the estimation of superoxide dismutase, lipid peroxidation, catalase, reduced glutathione and for histopathological studies.

Gentamicin administration showed an increase in lipid peroxidation and a decrease in superoxide dismutase, catalase and reduced glutathione levels. Administration of herbal extracts at various dose levels brought about a significant reduction in lipid peroxidation and an increase in the activities of antioxidant enzymes namely, superoxide dismutase, catalase and reduced glutathione which suggest its efficacy in alleviating the free radical-induced damage. Among *Hygrophila spinosa* and *Mangifera indica* treated groups, ethanolic extract of *Hygrophila spinosa* at 250 mg/kg predominantly reversed the changes caused by gentamicin administration.

Serum creatinine level is used as an index of nephrotoxicity. Serum creatinine level which was elevated following gentamicin administration was lowered significantly on $15^{th}$ day itself by ethanolic and aqueous extracts of *Hygrophila spinosa* at a dose rate of 250 mg/kg and also by ethanolic and aqueous extracts of *Mangifera indica* at a dose rate of 500 mg/kg. All the other plant extracts lowered serum creatinine on $30^{th}$ day. Among *Hygrophila spinosa* and *Mangifera indica* treated groups, the most effective treatment was found with that of ethanolic extract of *Hygrophila spinosa* at 250 mg/kg.

Serum urea levels were significantly lowered on $30^{th}$ day by the plant extracts and the mean values indicated that significant reduction was found with ethanolic extract of *Hygrophila spinosa* at 250 mg/kg and aqueous extract of *Mangifera indica* at 500 mg/kg. The most significant reduction was shown by ethanolic extract of *Hygrophila spinosa* at 250 mg/kg.

The serum albumin, total protein, sodium, potassium and calcium levels showed no significant reduction and they were found to be within normal range throughout the experiment.

The results substantiated by histopathological studies confirmed that treatment with *Hygrophila spinosa* and *Mangifera indica* alleviated the gentamicin-

induced proximal tubular necrosis, interstitial oedema, perivascular oedema and mononuclear cells infiltration. The regenerative changes were predominant with ethanolic extract of *Hygrophila spinosa* at 250 mg/kg.

Phytochemical analysis of plant extracts revealed the presence of steroids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes and saponins.

In the present study, *Hygrophila spinosa* and *Mangifera indica* showed a striking nephroprotective action in a dose-dependent manner by decreasing the serum creatinine and urea levels, and lessened the negative effects of gentamicin-induced nephrotoxicity possibly, by inhibiting free radical mediated process. The presence of some of the phytochemical constituents viz. flavonoids and phenolic compounds in the plant extracts may be the reason for the antioxidant effect of these extracts. The diuretic property of whole plant of *Hygrophila spinosa*, high in potassium content, may also be one of the contributing factors for the nephroprotection than treatment with the stem bark of *Mangifera indica.*

From the experiment, it is concluded that *Hygrophila spinosa* and *Mangifera indica* possess nephroprotective action and hence these plants can be recommended for the treatment of various nephrotoxicosis in animals and man.

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# NEPHROPROTECTIVE EFFECT OF *Hygrophila spinosa* AND *Mangifera indica* IN ALBINO RATS

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## ABSTRACT

The present study was undertaken to assess the nephroprotective effect of whole plant of *Hygrophila spinosa* and stem bark of *Mangifera indica* against gentamicin-induced nephrotoxicity in albino rats.

The experiment was conducted for a period of 30 days. The animals were divided into ten groups comprising eight each. Group I served as healthy control which received vehicle alone. Groups II, III, IV, V, VI, VII, VIII, IX and X received gentamicin sulphate at a dose rate of 80 mg/kg i.p. for eight days. Group II was retained as such without any treatment till the completion of the experiment. Group III and Group IV received ethanolic extracts of *Hygrophila spinosa* at a dose rate of 50 mg/kg p.o. and 250 mg/kg p.o. respectively whereas Group V and Group VI received aqueous extracts of *Hygrophila spinosa* at a dose rate of 50 mg/kg p.o. and 250 mg/kg p.o. respectively. Group VII and Group VIII received ethanolic extracts of *Mangifera indica* at a dose rate of 100 mg/kg p.o. and 500 mg/kg p.o. respectively while Group IX and Group X received aqueous extracts of *Mangifera indica* at a dose rate of 100 mg/kg p.o. and 500 mg/kg p.o. respectively. The blood samples were collected on $0^{th}$, $9^{th}$, $15^{th}$ and $30^{th}$ day and serum was used for the estimation of creatinine, urea, albumin, total protein, sodium, potassium and calcium. On $30^{th}$ day, all the animals were sacrificed and kidney was used for the estimation of superoxide dismutase, lipid peroxidation, catalase and reduced glutathione. Histopathological studies were also conducted to assess the nephroprotective action of these plant extracts.

Administration of the herbal extracts at various dose levels brought about a significant reduction in lipid peroxidation and an increase in the activities of superoxide dismutase, catalase and reduced glutathione, which suggest its efficacy in scavenging free radical-induced renal damage. Treatment with ethanolic extract of

*Hygrophila spinosa* at the dose rate of 250 mg/kg was found to be the most effective treatment in scavenging the free radical-induced gentamicin nephrotoxicity.

Treatment with the plant extracts showed a decreased creatinine level following gentamicin administration. The serum urea levels were significantly reduced in all the treatment groups. The significant reduction in serum urea levels was predominant in ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg and aqueous extracts of *Mangifera indica*. The most significant reduction in serum creatinine and serum urea levels was shown by ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg. Serum levels of albumin, total protein, sodium, potassium and calcium were within the normal range throughout the experiment.

The results were substantiated by histopathological studies, which confirmed that treatment with ethanolic and aqueous extracts of *Hygrophila spinosa* and *Mangifera indica* inhibited gentamicin-induced proximal tubular necrosis. Ethanolic extract of *Hygrophila spinosa* at the dose rate of 250 mg/kg was found to be most effective in favouring nephroprotection.

Phytochemical analysis of plant extracts revealed the presence of steroids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes and saponins.

The findings of the present study validate the nephroprotective effect of *Hygrophila spinosa* and *Mangifera indica* for the management of renal disorders.