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HEPATO AND NEPHROPROTECTIVE ACTIVITY OF ETHANOL EXTRACT OF *SEBASTIANA CHAMAELEA* ON PARACETAMOL INDUCED NEPHROTOXICITY IN MALE WISTAR RATS

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ABSTRACT

To investigate the hepato and nephroprotective activity of ethanol extract of *Sebastiana chamaelea* on Paracetamol induced nephrotoxicity in male Wistar rats. In this model of nephrotoxicity, 30 adult male wistar rats (150-200gms) were evenly divided into 5 groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 2 0 0 and 400 mg/kg e x t r a c t respectively, after each dose of Paracetamol (200 mg/kg, i.p. for 3 days) from4 to 14 days . On 11th day, blood samples for biochemical parameters, while the rats kidneys for histology were obtained under inhaled diether anaesthesia. Paracetamol treatment caused hepato a n d nephrotoxicity as evidenced by marked elevation in blood urea, uric acid and creatinine, bilirubin. Co-administration of e x t r a c t with Paracetanmol decreased rise in blood urea, uric acid and creatinine, bilirubin. Apart from these, histopathological changes also showed the protective nature of extract against Paracetamol induced necrotic and hepatic damage of renal and hepatic tissues. It was observed that the ethanol extract of conferred nephroprotective and hepatoprotective activities by histopathological and biochemical observation against Paracetamol induced nephrotoxicity and hepatotoxicity in rats. In the near future could constitute a lead to discovery of a novel drug for treatment of drug induced nephrotoxicity and hepato toxicity.

KEY WORDS: *Sebastiana chamaelea*, Paracetamol induced nephrotoxicity, hepato and nephroprotective activity

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INTRODUCTION

Kidneys have some delicate tasks, especially when they have to deal with unwanted substances, which they have to clear from the system, especially toxins. On top of this they play an important part in the maintenance of our endocrine and acid-base balance, blood pressure, erythropoiesis (creation of new red blood cells) etc., a real multi-tasking unit inside our body, which comes in pairs (a dual core processor by mother nature). Therefore it becomes critical when kidney functions decline, induced by diseases which seem to have no direct relation to renal pathophysiology. Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and

medication, on the kidney. There are various forms of toxicity. Nephrotoxicity should not be confused with the fact that some medications have a predominantly renal excretion and need their dose adjusted for the decreased renal function (e.g. heparin).Several drugs are nephrotoxic. Reactions to drugs and other compounds are relatively common and have been described for many substances. They are commonly associated with renal dysfunction although the actual incidence of drug-induced renal failure has not been reported, since incidence is complicated by the complexity of the causes of ARF in seriously ill patients. Nephrotoxicity arises through several mechanisms, including general and local vascular effects, direct effects on renal tubules, tubular obstruction and acute interstitial nephritis. Acute glomerulonephritis can also occur although this is less common.

The incidence of nephrotoxicity from aminoglycosides has increased from 2 to 3% in 1969 to 20% in the past decade. Despite nephrotoxicity and ototoxicity, the aminoglycosides are continuously being used in clinical practice because of their bactericidal efficacy, synergism with ß-lactam agents, low cost, limited bacterial resistance, and a post-antibiotic effect. Nephrotoxicity has been recognized as a major complication of aminoglycoside antibiotics for many years. During the past 6 to 8 years, this problem has attracted the attention and interest of a number of investigators, resulting in the generation of a large body of experimental data that has greatly expanded our understanding of the pathogenesis of this disorder. The human beings are exposed to environmental, occupational and xenobiotics challenges due to modern life style. Enormous free radicals are generated during the exposure to such stressful challenges. In addition the process of metabolism and excretion of xenobiotics may also generate free radicals. These free radicals bind covalently with the tissue macromolecules leading to the cell necrosis. Paracetamol is a safe and effective analgesic and antipyretic. It is widely available as a single-component medication and also as a component of a plethora of combination over-the-counter and prescription medications. More than 28 billion doses of Paracetamol-containing products were dispensed in 2005. With more than 89 million prescriptions, hydrocodone/Paracetamol was the most commonly dispensed medication in 2003. Despite its safety when used properly, Paracetamol is one of the more common overdoses reported to poison centers. Serious toxicity results in hepatic injury, which may progress to fulminant hepatic failure(FHF) and death. In 2009, the American Association of Poison Control Centers' National Poison Data System reported 401 deaths caused by Paracetamol or an Paracetamol combination product. Paracetamol is the most common cause of acute liver failure (ALF) in the United States, accounting for nearly half of the cases of ALF in the US Acute Liver Failure Study Group. Additionally, a significant number of Cases of ALF of unknown cause may be unrecognized Paracetamol toxicity, suggested by the presence of Paracetamol protein adducts. In children, Paracetamol is much less frequently the cause of acute liver failure (1-12).

Traditional healers and pharmacists in developing countries are in important source of information about plant sources of new drugs. Only a fraction of the earth's natural pharmacopoeia has been analyzed with modern techniques. The threat of imminent extinction of many plant species, especially in tropical areas, makes it urgent that scientists learn as much as possible before old remedies are forgotten or their raw materials are destroyed. This process requires the observation and recording of medical techniques, identification of plant materials and experimental investigation of the ingredients and their effects. Ethnopharmacology can also be an important element of a developing nation's medical and economic system. Third World governments are being encouraged to seek a synthesis between modern and traditional medicine. Although developing countries are providing many of the raw materials needed in drug manufacturing, the final products are often returned as high-priced medicines. As more plants are needed for large-scale production, over harvesting has led to stock depletion.

Chemists have so far been unable to reproduce the complex structure of many plant compounds. Further coordinated research into folk traditions, plant species,

growing conditions and local medical needs is urged. care must be taken however to preserve the main advantages of traditional medical care, low cost and easy access. Now-a-days natural products are an integral part of human health care system, because there is popular concern over toxicity and resistance of modern drugs. India is one of the 12 leading biodiversity centers with presence of over 45,000 different plant species, 15000-18000 flowering plants, 23,000 fungi, 16,000 lichens, 18,000 bryophytes and 13 million marine organisms. From this flora, 15,000 to 20,000 have good medicinal value. Among those only about 7,000 plants are used in Ayurveda,600 in Siddha, 700 in Unani and 30 in modern medicines. Liver is the heaviest gland of the body weighing about 1.4 kg in an average adult and is inferior to the diaphragm occupying most of the right hypochondriac and a part of the epigastric region of abdominopelvic cavity. The liver is divided into right and left lobe constituted by hepatocytes which are arranged in irregular, branching interconnected plates around a central vein. The liver also consists of sinusoids through which the liver receives the blood. The sinusoids consists of fixed phagocytes called stellate reticuloendothelial (kupffer) cells which destroy the worn out white blood cells, red blood cells, bacterial and other foreign matter in the venous blood draining from the gastrointestinal tract. Bile is partially excretory product and partially a digestive secretion from hepatocytes. The sodium and potassium salts of bile acids play an important role in emulsification and breakdown of large lipid globules into a suspension of droplets and also in the absorption of lipids following their digestion.

According to WHO about 18,000 people die every year due to liver diseases. The common ailments of liver are cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, fulminant hepatic failure and certain tumors like hepatoma. It is estimated that two billion people around the world are infected with hepatitis B. About 350 million of these have the chronic form of the disease. This alarming statistics with perplexing report, warrant the immediate necessity of studies of any level to either ensure the effectiveness of available formulations or exploration of the new herbal therapies to reduce the morbidity and mortality rate due to hepatic complications. In modern medicine cortico steroids and immunosuppresents are commonly used to treat liver disease in allopathic form of medicine. But, these drugs are associated with adverse effects such as immunosuppression and bone marrow depression. Further, the success rate of treating liver diseases is disappointing. Attempts are being made globally to get scientific evidences for this traditionally reported herbal drugs. In view of severe undesirable side effects of synthetic agents and absence of reliable liver protective drugs in the modern medicine, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the use of traditional herbal medicines which are claimed to possess hepatoprotective activity About 70-80% of the world populations rely on the use of traditional medicine, which is predominantly based on plant materials. The traditional medicine refers to a broad range of natural health care practices including Ayurveda, Siddha, Homeopathy and Unani.

The main objective of the study is to evaluate the nephro and hepatoprotective activity of the ethanolic extract of the *Sebastiania chamaelea* in validated experimental animal models.

MATERIALS AND METHODS (10, 11)

Collection of plant material

The *Sebastiania chamaelea* used for the present studies was collected from Chitoor district of Andhra pradesh. The plant was identified, confirmed and authenticated by comparing with voucher specimen available at Survey of medicinal plants & collection unit, Department of Botany, Sri Venkateswara University, Tirupathi by Field Botanist Dr. Madhav shetty. The bark was cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanichal grinder. The resulting powder was then used for extraction.

Preparation of Ethanolic Extract

The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 1500 ml of methanol for seven days. The extract was

concentrated and dried using Rotary flash evaporator. It was kept in dessicator until used.

Experimental Animals

Albino rats (Wistar strain) of either sex weighing between 150-200 g were procured from the Sai nath Agencies .zed for seven days under laboratory conditions. The animals were fed with commercially available rat pelleted diet (Sai Durga feeds & foods, Bangalore).Water was allowed *ad libitum* under strict hygienic conditions. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) (Approval IAEC/869/11-12) studies were performed in accordance with the CPCSEA guidelines.

Preparation of dose

Ethanolic extract of Sebastiania chamaelea was suspended in 3% CMC, to prepare a dose of 2000 mg/kg body weight of animal, and administered 1ml/100gm body weight of the animal.

Procedure

The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Two set of healthy female rats (each set of 3 rats) were used for the experiment. First set animals were divided and fasted for 18 hours deprived from food, water withdrawn before 4 hours of the dosing, body weights were noted before and after dosing with Ethanolic extract of *Sebastiania chamaelea* (2000 mg/kg) orally. Individually animals were observed for 4 hours to see any clinical symptoms, any change in behaviour or mortality. 6 hours post dosing again body weights recorded. From the next day onwards, each day for 1 hour the behavioural change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights wererecorded on 8^{th} and 14^{th} day. The same procedure was repeated with another set of animals to nullify the errors.

Effect of *Sebastiania chamaelea* on Aetaminophen-induced Hepato and nephrotoxicity

Experimental design:

Rats were divided into five groups, each group consisting of six animals. Group 1: Control with 2% tween 80; Group 2: Acetaminophen (500 mg/kg/body weight, p.o.), daily for 3 days108; Group 3:ethanol extract of *Sebastiania chamaelea* (200mg/kg/body weight,p.o) and simultaneously administered Acetaminophen (500 mg/kg/bodyweight, p.o.), daily for 3 days(induction) and 4 to 14 days (treatment); Group 4:ethanol extract of *Sebastiania chamaelea* (400mg/kg/body Weight, p.o.) And simultaneously administered Acetaminophen (500 mg/kg/body weight, p.o), daily for 3 days(induction) and 4 to 14 days(treatment); Group 5: Silymarin (25 mg/kg/body Weight, p.o.) and simultaneously administered Acetaminophen (500 mg/kg/body weight, p.o.), daily for 3 days (induction) and 4 to 14 days (treatment). At the end of experimental period, all the animals were sacrificed under diethyl ether anesthesia. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of kidney function

Biochemical parameters i.e., Estimation of Blood urea, Creatinine105-106 and uric acid107 SGOT,SGPT,ALP,bilirubin were analyzed according to the reported methods.The kidney and liver were removed, weighed and morphological changes were observed. A portion of kidney and liver were fixed in 10% formalin for histopathological studies.

Statistical analysis

The values were expressed as Mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnets test multiple comparison test. P values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

The use of Acetaminophen, a antipyretic and analgesic is equally associate with Hepato and nephrotoxicity as its side effect. Thus Acetaminophen induced nephrotoxicity and hepatotoxicity is well established experimental model of drug induced hepato and renal injury. Many animal experiments have demonstrated overwhelmingly, the positive correlation between oxidative stress and nephrotoxicity. Acetaminophen

induced Hepato, nephrotoxicity by causing renal phospholipidosis through inhibition of lysosomal hydrolases such as sphingomylinase and phospholipases in addition to causing oxidative stess. Drug induced nephrotoxicity are often associated with marked elevation in blood urea, serum creatinine and acute tubular necrosis. So these biochemical parameters have been used to investigate drug induced nephrotoxicity in animal and man. In the present study drug induced nephrotoxicity were established by daily administration of the Acetaminophen, for 3 days. This toxicity characterized by marked elevation in the circulating levels of blood urea, serum creatinine and histological features of tubulonephritis in the model control (group 2) rats when compared to untreated (group 1) rats. However these changes were attributed by pretreatment with single daily graded doses of ESC extract for 14 days. Oral administration of plant extract significantly decreases the urea and creatinine level in both treatment group compare to toxicant group. Apart from the direct nephrotoxic effect of Acetaminophen in group 2 rats, the acute elevation in the measured biochemical parameters could also be attributed to increased catabolic state of the rats due to the prolong anorexia associated with Acetaminophen nephrotoxicity. In renal diseases, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. Elevation of urea and creatinine levels in serum was taken as the index of nephrotoxicity. Creatinine derives from endogenous sources by tissue creatinine breakdown. Thus serum urea concentration is often considered a more reliable renal function prediction than serum creatinine. Anyhow the level of uric acid is nonsignificantly increased in the toxicant group when compared to control. Oral administration of plant extract significantly decreases the uric acid level in both treatment group compare to toxicant group. It was established that Acetaminophen is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR (Table-1).

Table-1 Effect of 500 mg/kg/day Oral Acetaminophen and *Sebastiania chamaelea* leaves oral on serum creatinine; blood urea and serum uric acid in treated rats for 14 days

Group	Drug treatment	Serum creatinine	Blood urea (mg/dl)	Uric acid
1	2% tween 80, p.o.,	0.29±0.01	21.59±3.73	2.35±0.12
2	500 mg/kg p.o, Acetaminophen	0.96±0.04***	118.76±5.981***	8.72±0.21
3	500 mg/kg p.o, Acetaminophen+Silymarin 25 mg/kg	0.43±0.01** *	44.26±4.20***	5.35±0.11* **
4	500 mg/kg p.o, Acetaminophen+200 mg/kg	0.82±0.01***	59.86±5.1** *	6.95±0.17***
5	500 mg/kg p.o, Acetaminophen+400 mg/kg	0.52±0.01***	47.76±3.2***	6.15±0.24***

N=6 animals in a group; Values are expressed as Mean ± SEM;

*: $p<0.05$, **$p<0.01$, *** $p<0.001$ vs Normal Control. ns indicate no significant.

In histopathological study of Normal group showing some blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. Acetaminophen treated group showing diffuse glomerular congestion, Tubular casts, Peritubular congestion, epithelial desquamation, Blood vessel congestion. While treatment group (200 mg/kg, Group III) shows Focal

glomerular congestion, Peritubular congestion, Focal hydrophic degeneration of tubular epithelial cells and treatment group (400 mg/kg, Group IV) shows only some of the blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. From histopatological results we can conclude that M.E.A.R extract at dose of 200 mg/kg have partial protective effect while ESC extract at dose of 400 mg/kg have protective effect on Acetaminophen induced nephrotoxicity. The findings suggest the potential use of methanol extract of ESC a therapeutically useful nephroprotective agent. Therefore further studies to explain their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases. Hepatotoxin gets converted into radicals in liver by action of enzymes & these attacks the unsaturated fatty acids of membranes in presence of oxygen to give lipid peroxides consequently. The functional integrity of hepatic mitochondria is altered, leading to liver damage. During hepatic damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in increased concentrations. Acetaminophen administration for 3 days significantly increased all these serum enzymes. Serum levels of SGPT can increase due to damage of the tissues producing acute hepatic necrosis, such as viral hepatitis and acute cholestasis (Table-2).

Table-2 Effect of 500 mg/kg/day Oral Acetaminophen and *Sebastiania chamaelea* leaves oral on **SGOT, SGPT, ALP in treated rats for 14 days**

Group	Treatment	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)
1	2% tween 80, p.o.,	31.8±1.37	40.87±1.49	28.78±1.62
2	500 mg/kg p.o,Acetaminophen	105.87±1.69***	128.91±3.33***	86.02±2.68***
3	500 mg/kg p.o, Acetaminophen+Silymarin 25 mg/kg	51.26±0.91***	50.64±1.35**	47.02±1.95***
4	500 mg/kg p.o, Acetaminophen+200 mg/kg	72.17±2.02***	76.88±1.41***	59.86±1.42***
5	500 mg/kg p.o, Acetaminophen+400 mg/kg	60.4 9±1.36***	53.07±1.94***	50.47±1.58***

N=6 animals in a group; Values are expressed as Mean ± SEM;
*: $p<0.05$, **$p<0.01$, *** $p<0.001$ vs Normal Control. ns indicate no significant.

Acetaminophen induced liver damage and alcoholic cirrhosis also can associate with mild to moderate elevation of transaminases. In the current study treatment of rats with ethanolic extract of leaves of Sebastiania chamaelea significantly($p<0.05$ in 200mg/kg b.w. and $p<0.01$ in 400mg/kg b.w.) decreased the levels of SGPT in serum which is an indication of hepatoprotective activity. SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT levels in serum due to the damage to the tissues producing acute necrosis, such as severe viral hepatitis & acute cholestasis. Alcoholic liver damage and cirrhosis can also associate with mild to moderate elevation of transaminase. In the current study treatment of animals with methanolic extract of leaves of Sebastiania chamaelea significantly($p<0.05$) decreased the levels of SGOT in serum which is an indicative of hepatoprotective activity. In case of toxic liver, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells. In the current study treatment of animals with methanolic extract of leaves of *Sebastiania chamaelea* significantly($p<0.05$ in 200mg/kg b.w. and $p<0.001$ in 400mg/kg b.w) decreased the

levels of ALP in serum as an indication of hepatoprotective activity. In case of toxic liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin. Such a situation can occur in generalized liver cell injury. Certain drugs (e.g., rifampin and probenecid) interfere with the net uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia92. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert's disease.

In the current study treatment of animals with ethanolic extract leaves of *Sebastiania chamaelea* significantly ($p<0.05$ in 200mg/kg b.w. and $p<0.01$ in 400mg/kg b.w) decrease the levels of bilirubin (direct and total) in serum which is an indication of hepatoprotective activity (Fig-1 and 2).

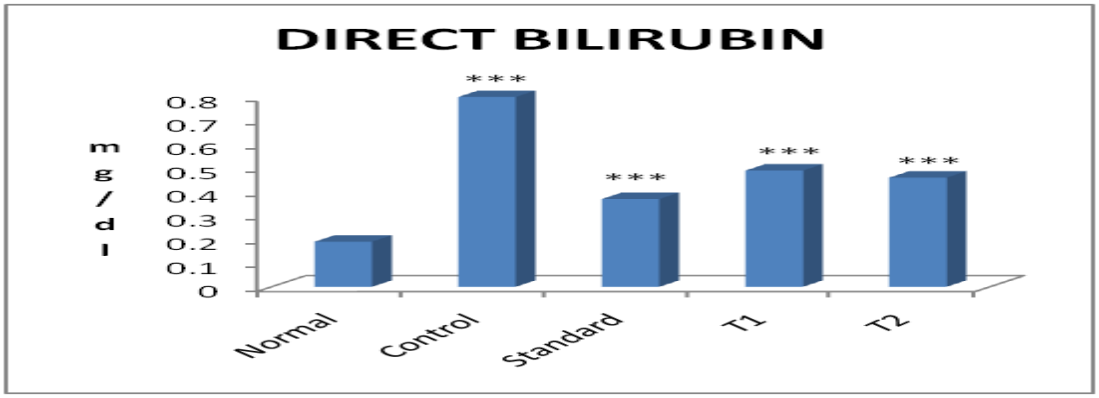

Figure-1 Effect of extract on biochemical parameter Direct Bilirubin

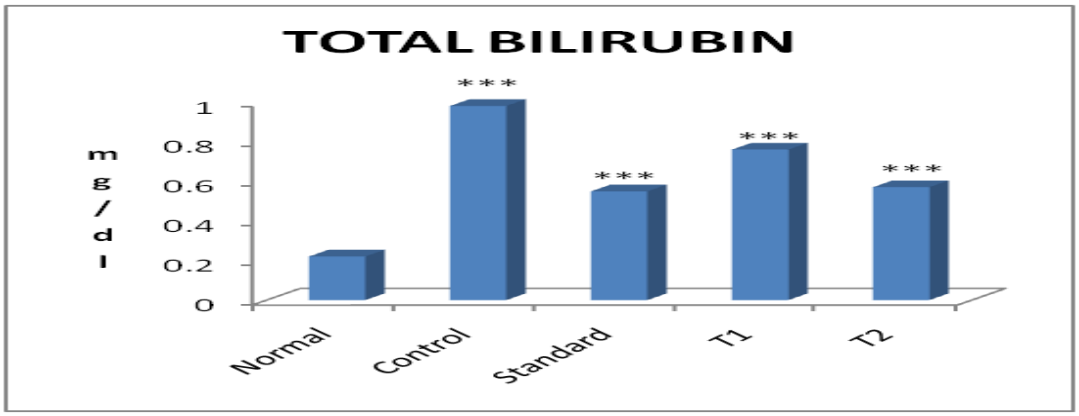

Figure-2 Effect of extract on biochemical parameter Total Bilirubin

The LOD for this method was found to be 4.61µg/ml and 14.0 µg/ml for etizolam. The LOQ for this method was found to be 0.21µg/ml for escitalopram oxalate and 0.63µg/ml for etizolam.

CONCLUSION

In the present study, the extract of *Sebastiania chamaelea* treated group animals were found to reduce such changes in kidney histology in toxicity induced by Acetaminophen, indicating nephroprotection. Further documented reports reveal that, plant material containing phenols, flavonoids, alkaloids and saponins offers organ protection by virtue of their free radical scavenging activity. The extract under study upon phytochemical analysis showed the presence of a fore mentioned phytoconstituents. Hence, the role of these

phytoconstituents as free radical scavengers and consequent nephroprotection cannot be ruled out. Acetaminophen induced hepatotoxicity was significantly prevented by pretreatment with ethanolic extract of *Sebastiania chamaelea*. Reduction in elevated biochemical parameter levels like serum SGPT, SGOT, ALP, direct and total bilirubin, after treatment with methanolic extract of *Sebastiania chamaelea* confirmed the hepatoprotective effect of extract under study. In liver injury models in rats restoration of hepatic cells with minor fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepatoprotection. Based on improvement in serum marker enzyme levels, functional parameters and histopathological studies it was concluded that ethanolic extract of *Sebastiania chamaelea* possesses significant hepatoprotective activity in the doses used.

REFERENCES

1. Leehey DJ, Braun BI, Tholi DA. Can pharmacokinetic dosing decrease nephrotoxicity associated with aminoglycoside therapy. *J Am Soc Nephrol* 1993;4: 81-90.
2. George JK, Enrique PM. Aminoglycoside nephrotoxicity. *Kidney Int* 1980; 8:571-582.
3. Vijay KK, Naidu MUR, Shifow AA, Ratnakar KS. Nephroprotective and antioxidant activity of ehanolic extract of the bark of Madhuca longifolia (koenig). *Indian J Pharmacol* 2000; 32: 108.
4. Temple AR, Lynch JM, Vena J, et al. Aminotransferase activities in healthy subjects receiving three-day dosing of 4, 6, or 8 grams per day of acetamino-phen. *Clin Toxicol (Phila)* 2007;45(1):36–44.
5. FDA. *Acetaminophen overdose and liver injury _ background and options for reducing injury.*
6. Wysowski DK, Governale LA, Swann J. Trends in outpatient prescription drug use and related costs in the US: 1998-2003. *Pharmacoeconomics* 2006;24(3): 233–6.
7. Prescott LF, Roscoe P, Wright N, et al. Plasma-paracetamol half-life and hepatic necrosis in patients with paracetamol overdosage. *Lancet* 1971;1(7698):519–22.
8. Bronstein AC, Spyker DA, Cantilena LR Jr, et al. 2009 Annual report of the Amer-ican Association of Poison Control Centers' National Poison Data System (NPDS): 27th annual report. *Clin Toxicol (Phila)* 2010;48(10):979–1178.
9. Ostapowicz G, Fontana RJ, Schiødt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med* 2002;137(12):947–54.
10. FDA. Drug induced liver toxicity. Available at: http://www.fda.gov/Drugs/ ScienceResearch/ResearchAreas/ucm071471.htm. Accessed October 8, 2011.
11. Davern TJ II, James LP, Hinson JA, et al. Measurement of serum acetaminophen-protein adducts in patients with acute liver failure.*Gastroenterology* 2006;130(3): 687–94.
12. Squires RH Jr, Shneider BL, Bucuvalas J, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. *J Pediatr* 2006; 148(5):652–8.
13. K. S. Gopi, A. Gopala Reddy, K. Jyothi, and B. Anil Kumar Acetaminophen-induced Hepato- and Nephrotoxicity and Amelioration by Silymarin and Terminalia chebula in Rat. *Toxicol Int*. 2010 Jul-Dec; 17(2): 64–66.
14. 103. Word Health Organization 2000, Guidelines on Standard Operating Procedures For Clinical Chemistry. pp 69-73
15. Bowers LD. *Clin Chem*1980; 26: 551.