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EVALUATION OF HEPATO-PROTECTIVE ACTIVITY OF SEDESTIANA CHAMAELEA EXTRACT AGAINST PARACETAMOL INDUCED HEPATO- TOXICITY IN WISTAR RATS

K.Sivaji*, M.Sneha, CH.Satya Surya, A.Neelu Varalakshmi, V.Vidya, K.Sirisha, N.Naga Lakshmi, K.Hima Bindu

Department of Pharmacology, JITS College of Pharmacy, Kalgampudi, Andhra Pradesh, India.

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, 200 and 400 mg/kg extract respectively, after each dose of Paracetamol (200 mg/kg, i.p. for 3 days) from4 to 14 days . On 11^{th} 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 e x t r a c t 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.

Keywords: extract of *Sebastiana chamaelea*, nephrotoxicity and hepato toxicity

Author for correspondence
K.Sivaji,
Department of Pharmacology,
JITS College of Pharmacy, Kalgampudi,
Andhra Pradesh, India.
Email id: siva.bpharm09@gmail.com

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[1]. 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[5.] 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[7]. In 2009,the American Asso-ciation of Poison Control Centers' National Poison Data System reported 401 deaths caused by Paracetamol or an Paracetamol combination product[8]. 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 (1-5).

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

Collection of plant material (6-9)

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

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

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[103-104], 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 tests. P values <0.05 were considered as significant.

RESULTS AND DISCUSSION

In Acetaminophen treated group of animals the concentration of serum urea and creatinine were considerably increased than the normal animals (group 1) which indicates severe nephrotoxicity. Treating (group 4 & 5) with ethanol extract of showed significant decrease ($p<0.001$) in concentration of serum urea and creatinine compared to Acetaminophen treated group 2. Nevertheless the concentration of uric acid not so much considerably increased in the Acetaminophen treated groups (group 2) than control group (group1). Treatment with methanol extract of significantly ($p<0.05$) decreases the uric acid levels in group 4 & 5 ($p<0.01$) compared to Acetaminophen treated group (group 2) (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 (mg/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)
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+2 00 mg/kg	0.82±0.01***	59.86±5.1** *	6.95±0.17***
5	500 mg/kg p.o, Acetaminophen+4 00 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

Rats treated with Actaminophen developed a significant hepatic damage observed as elevated serum levels of hepato specific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with Silymarin, ethanolic extract had showed good protection against Actaminophen induced toxicity to liver. Test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals which are evident in table-2.

Table-2 Effect of 500 mg/kg/day Oral Acetaminophen and Sebastiania chamaelea leaves oral on SGOT, SGPT, and 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	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

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. 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, per tubular 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. 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.

CONCLUSION

In the present study, the extract of *Sebastiania chamaelea* significantly reduced the toxicant elevated levels of above mentioned serum markers and increase in the levels of protein. Hence, at this point it is concluded that the extract of Sebastiania chamaelea offers nephroprotection. In Acetaminophen treated animals there was glomerular, peritubular and blood vessel congestion and result in presence of inflammatory cells in kidney sections. The same is observed in case of humans who are suffering from major kidney disorders. 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.

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