Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(7):68-75

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Nephroprotective Effects of *Salacia fruticosa Heyne ex Lawson* Against Acetaminophen-Induced Nephrotoxicity and Oxidative Stress in Rats

D Rajalingam[1,2*] and S Palani[3]

[1]*Department of Pharmaceutical Chemistry, Kamalakshi Pandurangan College of Pharmacy Thiruvannamalai, Tamil Nadu, India*
[2]*Research Scholar, Manonmanium Sundaranar University, Tirunelveli, Tamil Nadu, India*
[3]*Department of Biotechnology, Anna Bioresearch Foundation, Arunai Engineering College, Tiruvannamalai, Tamil Nadu, India*

ABSTRACT

The present investigations were undertaken to evaluate the Nephroprotective effect of the ethanol extract of Salacia fruticosa Heyne ex Lawson against Acetaminophen-induced nephrotoxicity and oxidative stress in rats. Salacia fruticosa (Hippocrateacea) have been used in Ayurvedic, Siddha and Folklore for various diseases in India. The extent of renal damage and effect of ethanol extract of Salacia fruticosa (EESF) were assessed by various biochemical parameters in the experimental rats. Histopathological changes in the renal tissues of experimental rats were also studied. The administration of effect of ethanol extract of Salacia fruticosa at dose levels of 250 and 500 mg/kg/b.w orally were decreased in levels of blood Urea, uric acid, creatinine when compared to APAP treated rats. The antioxidant studies reveal that the rats treated with EESF were increased the levels of renal SOD, CAT, GSH and GPx, however the level of MDA were reduced as compared with APAP induced rats. Apart from these, histopathological changes also reveal that the protective nature of ethanol extract of Salacia fruticosa against acetaminophen induced necrotic damage of renal tissues. In conclusion, this study demonstrates that the protective effect of ethanol extract of Salacia fruticosa from APAP induced nephrotoxicity in rats and the results suggested that the possible mechanism of this effect may be due to free radical-scavenging and antioxidant activity.

Keywords: *Salacia fruticosa*; Acetaminophen; Nephroprotective; Antioxidant; Histopathalogy

---

INTRODUCTION

The kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs [1]. Therefore, the kidney can be considered as a major target organ for exogenous toxicants. Nephrotoxicity is a kidney-specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs [2,3]. The incidence of drug-induced nephrotoxicity has been increasing with the ever increasing number of drugs and with easy availability of over-the-counter medication i.e. non-steroidal anti-inflammatory drugs (NSAIDs). Antibiotics, angiotensin converting enzyme inhibitors (ACEI) and contrast agents are the major culprit drugs contributory to kidney damage [4].

Acetaminophen (APAP), also known as paracetamol, is the most widely used analgesic and antipyretic medication in the world that is safe at therapeutic dosages [5], APAP is known to cause hepatic necrosis and renal failure in both humans [6-8] and animals and can even lead to death [9,10] when administered in overdoses. Renal damage and acute renal failure can occur even in the absence of liver injury [8]. Several studies have shown that acetaminophen causes oxidative damage, including tissue lipid peroxidation, inhibitions of enzyme and reductions in glutathione levels and changes of enzymatic and non-enzymatic antioxidant systems [11]. Development of less

nephrotoxic drugs is challenging due to the fact that the prediction of nephrotoxicity during drug development remains difficult. Research are going on throughout the world for safe active molecules that would provide maximum protection to the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body [12,13]. A number of herbs are traditionally used in different countries during drug or toxin induced hepatic and renal disorders [14].

Salacia fruticosa Heyne ex Lawson belongs to the family Hippocrateaceae/Celastraceae, commonly known as Ponkarandi in Malayalam, Korandi in Tamil. It is a woody climbing shrub, distributed in South–West India (Karnataka, Tamilnadu, Kerala and Orisa) and Srilanka. In Tamilnadu, it is mostly seen in Dindugul and Kanniyakumari [15,16]. In traditional system of medicine, the plants of this genus are being used as acrid, bitter, thermogenic, urinary, astringent, anodyne, antiinflammatory, depurative, vulnerary, liver tonic and stomachic. The root of this plant is used for treating gonorrhea, rheumatism, obesity and skin diseases, antidiabetic, antihypertensive, hepatoprotective, urinary, anticaries and anticancer potentials [16-18]. It is one among the list of medicinal plants with proven antidiabetic and related beneficial effects and of herbal drugs used in treatment of Diabetes-[19]. There is no scientific data available to substantiate their Hepatoprotective and Nephroprotective activity. Therefore the present investigations are mainly emphasized on exploration and exploitation of the antioxidant and Nephroprotective potential of ethanol extract of roots of *Salacia fruticosa* against APAP-induced nephrotoxicity.

MATERIALS AND METHODS

Plant Material

The fresh of *Salacia fruticosa* Heyne ex Lawson was collected from Tirunelveli district of Tamilnadu, India, in the month of February. The plant was identified and authenticated by Dr. V. Chelladurai, Research officer, Botany C.C.R.A.S. Govt. of India, (Retired). The voucher specimen (KPCP4/2014), was deposited in our pharmaceutical chemistry laboratory for future reference. The whole plant was dried under shade, made into coarse powder with mechanical grinder, passed through 40 mesh sieves and stored in closed containers for further use.

Extraction Procedure

The dried, coarsely powdered root of *Salacia fruticosa* (500 g) was extracted with ethanol (90% v/v) in soxhlet apparatus at 60°C for 24 hours. Then the solvent was completely recovered on the ethanol extract of *Salacia fruticosa* (EESF) under reduced pressure by a rotary vacuum evaporator. The concentrated extract was dried on a water bath and preserved in a vacuum desiccator.

Chemicals

All reagents and drugs used were obtained commercially and were of analytical grade.

Animals

Studies were carried out using Wister albino male rats (180-200 g), obtained from Indian Veterinary Preventive Medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by the Poultry Research Station, Nandhanam, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of the experiment. All animal studies were approved by Institutional Animal Ethical Committee (IAEC) in accordance to the guidelines of CPCSEA.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD - 423 guidelines (acute toxic class method), Albino rats (n=6) of single sex were selected for the acute toxicity study. Which received a single oral dose of 2000 mg/kg body weight of ethanol extract of *Salacia fruticosa?* The dose was administered to overnight fasted rats and food was withheld for a further 3-4 hours after administration of the drug and observed for signs of toxicity for a period of 14 days [20].

Acetaminophen Induced Nephrotoxicity in Rats

After acclimatization the Rats were divided randomly into four groups (I–IV) of six rats in each group.

Group I Rats served as untreated control and was fed orally with normal saline 5 ml/kg body weight daily for 7 days.

Group II Rats (APAP only) were similarly treated as Group I.

Groups III and IV rats were treated with EESF 250 mg/kg and EESF 500 mg/kg for 7 days, respectively.
On the 7th day, acetaminophen suspension was given by oral route, in a dose of 750 mg/kg body weight [21] to all the groups of rats except the rats in Groups I.

Biochemical Parameters Assessed for Renal Function
After 48 h, animals were sacrificed by chloroform anesthesia. Blood samples were collected by cardiac puncher, using 21 gauge (21 G) needles mounted on a 5 ml syringe (Hindustan syringes and medical devices ltd, Faridabad, India.) The blood was centrifuged for 10 min at 5000 rpm. The obtained clear sera were stored at -20°C for subsequent measurement of urea (UR), creatinine (CR) and uric acid (UA) levels using colorimetric assay kits, Bayer (Seamon) according to the manufacturer's instructions.

Preparation of Renal Homogenate
The kidneys were removed and dissected free from the surrounding fat and connective tissue. Each kidney was longitudinally sectioned, and renal cortex was separated and kept at -8°C. Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for the determination of malondialdehyde (MDA) content, reduced glutathione (GSH) and antioxidant enzyme levels such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activity using colorimetric assay.

Biochemical Estimation of Markers of Oxidative Stress
MDA content was measured according to the earlier method reported [22]. SOD activity was determined according to the previous report [23]. CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method [24]. GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 [25]. Protein content in the tissue was determined by the method reported earlier (Lowry et al.) using bovine serum albumin (BSA) as the standard.

Histopathological Examination
Pieces of kidney section from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50–100%) alcohol, embedded in paraffin, cut into 4–5 μm thick sections and stained with hematoxylineosin [26]. The sections were evaluated for the pathological symptoms of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

Statistical Analysis
The results were expressed as mean ± SD of six animals from each group. One-way ANOVA followed by Dunnet tests have been used to analyze the data by Graph pad prism. $P<0.05$ was considered statistically significant.

RESULTS

Acute Toxicity
It was observed that the administration of single oral dose 2000 mg/kg/body weight of ethanol extract of *Salacia fruticosa* to a rat, didn't induce drug related toxicity and mortality in the animals and it was safe up to the dose of 2000 mg/kg/body weight

Effect of EESF on Biochemical Parameter
The effect of treatment with ethanol extract of *Salacia fruticosa* on APAP induced nephrotoxicity in rats was represented in the Figures 1 and 2. The animals administered only with APAP (Group II) resulted in a significant increase ($P<0.001$) in Serum urea, uric acid and creatinine concentrations level when compared to a normal control group (I). The toxic effects of APAP were controlled in the animals treated with ethanol extract of *Salacia fruticosa* at the doses of 250 and 500 mg/kg b.w., produced significant ($P<0.01$ and $P<0.001$) dose dependent decreases in serum urea (Figure 1), uric acid and creatinine (Figure 2) concentration level respectively as compared with normal control by the way of restoration of the level of the kidney function.

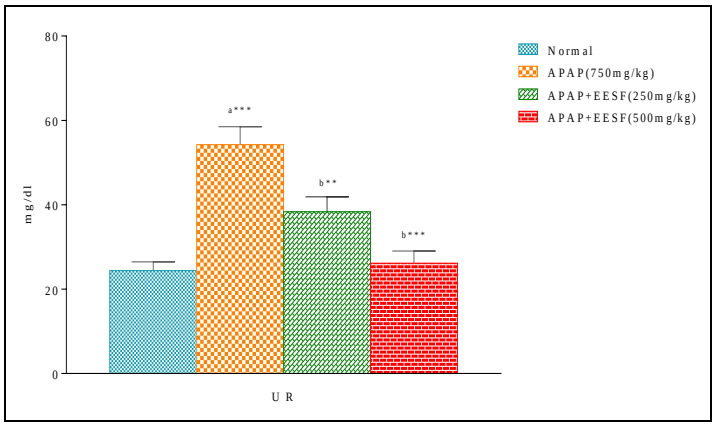


Figure 1: Effect of treatment with ethanol extract of *Salacia fruticosa* on the, blood Urea (UR; mg/dl)] level, in rats with APAP-induced nephrotoxicity

Values are expressed mean ± S.D for six rats in each group**, a** As compared with control, **b** As compared with APAP, ***represents P<0.001, **represents P<0.01.

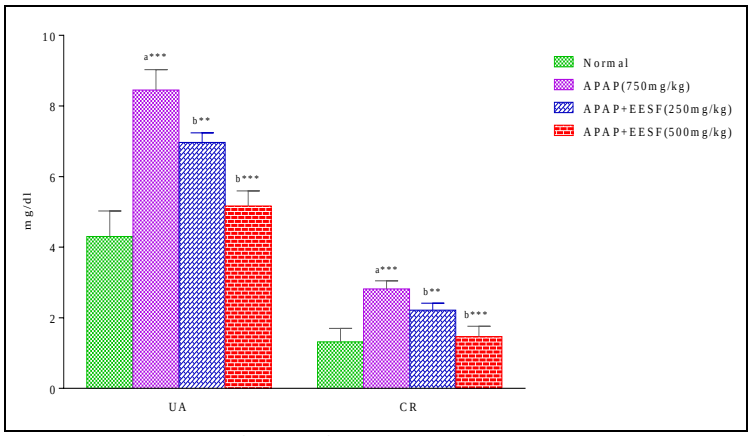


Figure 2: Effect of treatment with ethanol extract of *Salacia fruticosa* on the blood Uric acid (UA; mg/dl) and Creatinine (CR; mg/dl) level, in rats with APAP-induced nephrotoxicity

Values are expressed mean ± S.D for six rats in each group, **a** As compared with control, **b** As compared with APAP, ***represents P<0.001, **represents P<0.01.

Effect of EESF on Biochemical Oxidative Stress

The activity of Lipid peroxidation (LPO) level was Significantly (P<0.001) increased (Figure 3) and however the catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSH) (Figure 4) and superoxide dismutase (SOD) activity levels were Significantly (P<0.001) decreased (Figure 5) in rats treated with APAP (Group II), when compared with that of the normal control (Group I). Treatment of rats (Group III and Group IV) with ethanol extract of *Salacia fruticosa* at the dose of 250 and 500 mg/kg significantly (P<0.01, P<0.001) decreased the elevated lipid peroxidation levels and the decreased levels of catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase were restored to the normal levels in a dose dependent manner.

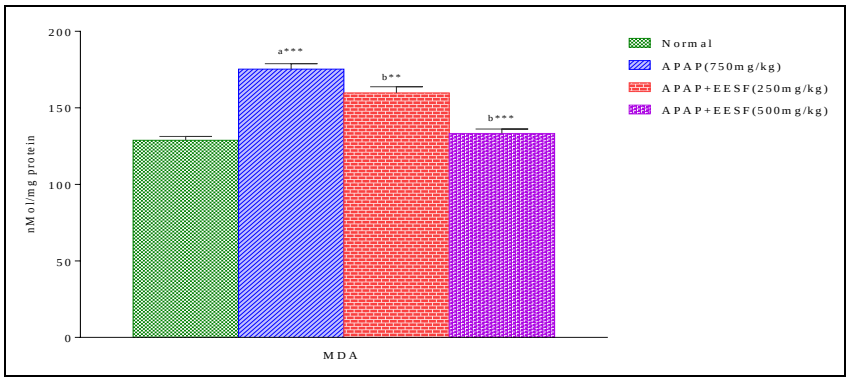


Figure 3: Effect of treatment with ethanol extract of *Salacia fruticosa* on renal MDA (nM/mg of protein) level in rats with APAP-induced nephrotoxicity

Values are expressed mean ± S.D for six rats in each group, **a** As compared with control, **b** As compared with APAP, ***represents P<0.001, **represents P<0.01.

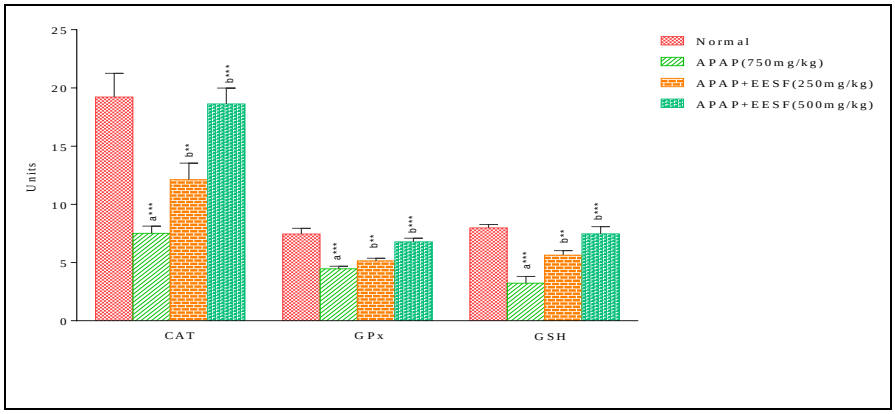


Figure 4: Effect of treatment with ethanol extract of *Salacia fruticosa* on the renal intracellular GSH activity (µg/mg protein), GPx (nmol of GSH oxidized/min/mg protein) and CAT (U/mg protein) in rats with APAP-induced nephrotoxicity

Values are expressed mean ± S.D for six rats in each group, **a** As compared with control, **b** As compared with APAP, ***represents P<0.001, **represents P<0.01.

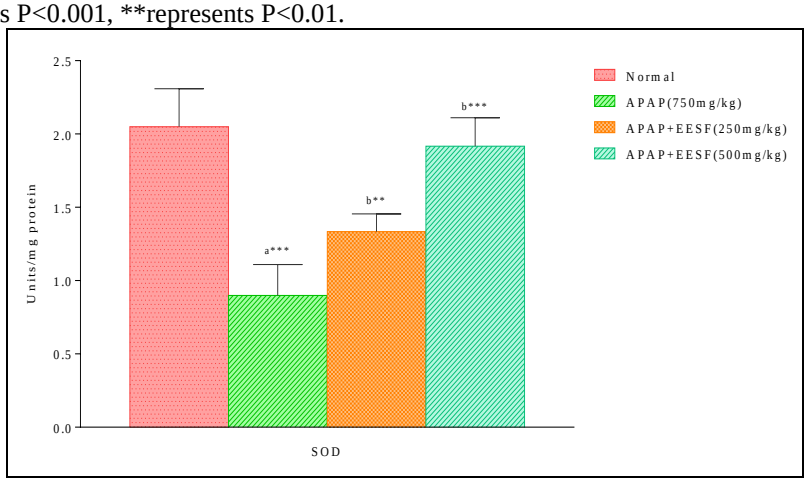
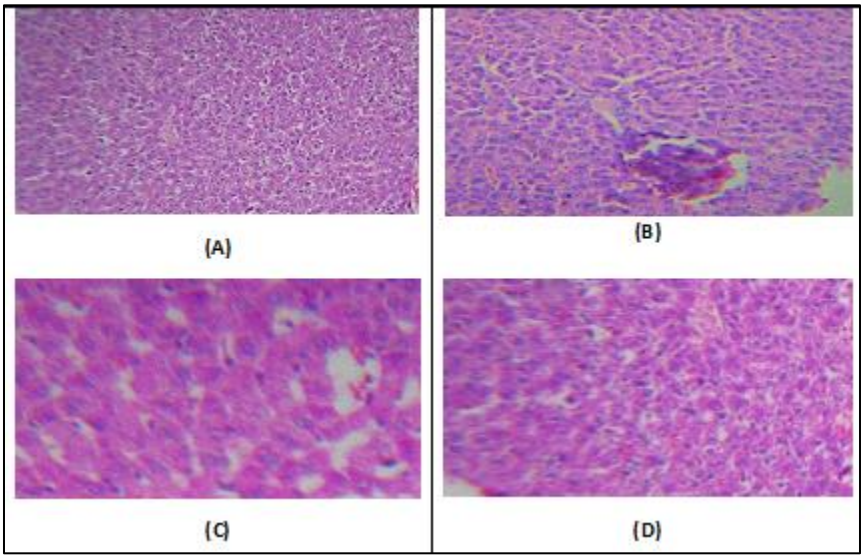


Figure 5: Effect of treatment with ethanol extract of *Salacia fruticosa* on renal SOD (units/mg protein) activity in rats with APAP-induced nephrotoxicity

Values are expressed mean ± S.D for six rats in each group, **a** As compared with control, **b** As compared with APAP, ***represents P<0.001, **represents P<0.01.

Histopathological Examination

The biochemical results were also confirmed by the histological pattern of normal kidney showing normal tubular brush borders and intact glomeruli and Bowman's capsule (Figure 6A). Treatments with acetaminophen shows sever tubular necrosis and degeneration in the renal tissue (Figure 6B). The rats treated with ethanol extract of SF (250 mg/kg body weight) showed normal tubular pattern with a mild degree of swelling, necrosis and degranulation (Figure 6C). Treatment with the ethanol extract of SF (500 mg/kg body weight) ameliorated the toxic manifestations in the kidney (Figure 6D).



(A) Normal control rats. (B) Acetaminophen (750 mg/kg) treated rats, (C) EESF 250 mg/kg + APAP treated rats (D) EEPA 500 mg/kg + APAP treated rats

Figure 6: Nephroprotective effect of ethanol extract of *Salacia fruticosa* (EESF) against APAP treated rats. Histopathological observations of kidney sections stained with Hematoxylin-Eosin (100×)

DISCUSSION

Various environmental toxicants and clinically useful drugs, like paracetamol and gentamicin, can cause severe organ toxicities through the metabolic activation to highly reactive free radicals including the superoxide and oxygen reactive species [27]. The selective renal accumulation of non-steroidal anti-inflammatory nephrotoxins including paracetamol in animal and human is thought to result in a chain of biochemical reactions which culminate in acute or chronic nephropathies [28]. In addition, paracetamol has been reported to promote hepatocyte and renal apoptosis [29,30]. Paracetamol toxic overdose is often manifested by too many metabolic and uric acid derangements. Serum urea and creatinine are considered the major nephrotoxicity markers [31], although serum urea concentration is often considered a more reliable renal function predictor than serum creatinine [32]. Blood urea nitrogen is found in the liver protein that is derived from diet or tissue sources and is normally excreted in the urine. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown [33].

In the present investigation, administration of a nephrotoxic dose of Acetaminophen to rats resulted in a significant ($p<0.01$) elevation of serum levels of urea, uric acid and creatinine in APAP treated group within 48 hours of exposure to it when compared with the normal control group. These results are in agreement with those observed in Isik B et al. [34], who noticed an elevation in serum urea and creatinine in rats after 1 g/kg body weight of paracetamol administration ,this elevation in the levels of urea and creatinine was explained by the presence of strong correlation between nephrotoxicity and oxidative stress [35,36]. However, daily pretreatment with ethanol extract of *Salacia fruticosa* for 7 days to the acetaminophen renal injured rats significantly ($P<0.001$) decreased the concentration levels of urea, uric acid, and creatinine to normal status in a dose dependent manner and 500 mg/kg dose offered maximum protection. Which proved the potency of renal cell regeneration capacity of *Salacia fruticosa.*

Several researches have shown that APAP- induced nephrotoxicity is associated with lipid peroxidation. This is attributed to a free radical-mediated chain reaction that damages cell membranes [37,38] and MDA is a good indicator of the degree of lipid peroxidation. In this study, we observed a significant increase in MDA levels in the renal tissue of rats treated with APAP alone compared with control. Administration of the plant extract inhibited the

increase in lipid peroxidation level in renal tissue. It is likely that the action of ethanol extract of *Salacia fruticosa* in reducing the membrane damage is mainly related to its ability to scavenge lipid peroxidation initiating agents.

Depletion of renal GSH is one of the primary factors that permit lipid peroxidation, it is suggested to be closely related to APAP tissue damage. It has been reported that renal glutathione content, and glutathione peroxidase and reductase activity of kidney tissue, which are critical constituents of the GSH-redox cycle, were significantly reduced by treatment with adriamycin, and the authors proposed that impairment of the kidney antioxidant defense mechanisms could permit enhanced free radical- induced kidney damage in adriamycin nephrotoxicity. Similarly, in the current study, administration of ethanol extract of *Salacia fruticosa* to APAP treated rats also increased the GSH level and the GSH-Px, CAT, and SOD activity of renal tissue. The increase in both non-enzymatic and enzymatic antioxidants might play a significant role in the mechanism of the Nephroprotective effect of *Salacia fruticosa.*

These findings can be further incorporated with histopathological studies. APAP induced renal damage is consistent with acute tubular necrosis. In the present study, the results of histopathological examination showed a clear evidence of nephrotoxicity following the administration of APAP in an overdose. Acute tubular necrosis was the most relevant histopathological change. These results are in agreement with those of the previous investigation describing the renal histological alterations following the administration of APAP in an overdose [39]. Treatment with ethanol extract of *Salacia fruticosa* ameliorated the APAP induced histopathological renal changes.

It is well established that medicinal plants with nephroprotective properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids and alkaloids they contain [40,41]. Literature review reveled that *Salacia fruticosa* contains abundant amount of phytoconstituents in which, Root contains Friedelan-3-one-29al, friedelan-3-one-29ol, friedelin, friedel-1-en-3-one, amyrin, Sitosterol, Salacinol, kotalanol, kotalagenin-16 acetate, magniferin, epicatechin, glycosidal tannins, triterpines, hydroxy ferruginol, lambertic acid [17,42]. Thus the nephroprotective activity of ethanol extract of *Salacia fruticosa,* may be attributed due to the presence of these constituents.

CONCLUSION

In conclusion, the present study demonstrated that the Ethanol extract of *Salacia fruticosa* possesses dose dependent strong antioxidant activities and significant protective effect against acute nephrotoxicity induced by APAP. The histopathological studies also substantiate the activity of the EESF. Therefore the results suggested that the possible mechanism of this activity may be due to free radical-scavenging and antioxidant activity. This result scientifically supports the usage of the plant in traditional medicine for the treatment of renal disorders. Further investigations are ongoing to find out the active molecule responsible for the activities in our laboratory.

REFERENCES

[1] MA Ferguson; VS Vaidya; JV Bonventre. *Toxicology*. **2008**, 245,182-193.
[2] W Finn; G Porter. Clinical Nephrotoxins, 2nd edition, Kluwer Academic Publishers, Massachusetts, **2003**; 621-655.
[3] HF Galley. *JR Col Surg Edinb.* **2000**, 45, 45-50.
[4] NP Singh; A Ganguli; A Prakash. *J Assoc Phys India.* **2003,** 51, 970-977.
[5] S Kanno; A Tomizawa; T Hiur; Y Osanai; M Kakuta; Y Kitajima; K Koiwai. *Biol Pharm Bull*. **2006**, 29(3), 472-476.
[6] JG O'Grady. *J Hepatol*. **1997**, 26, 41-46.
[7] LR Presscott; N Wright; P Roscoe; SS Brown. *Lancet*. **1971**, 1, 519-522.
[8] L Eguia; BJ Materson. *Pharmacotherapy*. **1997**, 17, 363-370.
[9] WZ Potter; DC Davis; JR Mitchell; DJ Jollow; JR Gillette; BB Brodie. *J Pharmacol Exp Therap*. **1973**, 187(1), 203-210.
[10] W Moller-Hartmann; CP Seigers. *J Appl Toxicol*. **1992**, 11, 141-146.
[11] E Ozcelik; S Uslu; D Burukoglu; A Musmul. *Pharmacogn Mag*. **2014,** 10, S217-S224.
[12] P Montilla; M Barcos; MC Munoz; I Bujalance; JR Munoz-Castaneda; I Tunez. *J Biochem Mol Biol.* **2005,** 38, 539-544.
[13] HH Mansour; HF Hafez; NM Fahmy. *J Biochem Mol Biol*. **2006**, 39, 656-661.
[14] HA El-Beshbishy. *J Biochem Mol Biol*. **2005**, 38, 300-306.
[15] Flora of India, Data base, published by Botanical Survey of India, **2000**, 5,153-154.
[16] Ramiah; Nair. *Res Indian Yoga Homoeopathy*. **1978**, 13, 2.

[17] MP Padmaa; JP Leena; TS Angelin. *J Nat Remed*. **2008**, 8(2), 116-117.
[18] PK Warrier; VPK Nambiar; C Ramankutty. Indian Medicinal Plants a Compendium of 500 Species, reprint, Orient Longman Ltd, Hyderabad, **1996**; 5, 47.
[19] S Chakravarty; JC Kalita. *Int J Sci Adv Technol*. **2011**, 1(7), 179-180.
[20] OECD, Acute oral toxicity. Acute oral toxic class method guideline 423 In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals OECD, Paris, June, **2000.**
[21] S Palani; S Raja; R Naresh; B Senthil Kumar. *Toxicol Mech Method*. **2010**, 20(4), 213-221.
[22] XZ Zhang. Crop Physiology Research Methods. Agricultural Press, Beijing, China. **1992**; 131-207.
[23] S Rai; A Wahil; K Mukherjee; BP Saha; PK Mukherjee. *J Ethnopharmacol*. **2006**, 104, 322-327.
[24] HU Bergmeyer, K Gowehn, H Grassel. In: Methods of Enzymatic Analysis, ed. HU Bergmeyer, **1974**, 438-439.
[25] DG Hafemann; RA Sunde; WG Houestra. *J Nutr*. **1974**,104, 580-584.
[26] JD Valeer. *J Hepatol*. **2003**, 39, S43-S49.
[27] P Abraham; G Wilfred. *Clinical Chim Acta*. **1999**, 289, 177-179.
[28] RG Schnellman. Casarett and Doull's Toxicology, The Basic Science of Poisons, 6th edition, McGraw-Hill Medical Publishing Division, New York, **2001**; 491-514.
[29] SD Ray; N Jena. *Arch Toxicol*. **2000**, 73, 594-606.
[30] AH Boulares; A Zoltoski; A Yakovlev; FJ Contreras; ME Smulson. *J Biol Chem.* **2002**, 277, 372-378.
[31] RD Adelman; WL Spangler; F Beasom; G Ishizaki; GM Conzelman. *J Antimicrob Chemotherap*. **1981**, 7(4), 431-440.
[32] S Palani; S Raja; RP Kumar; S Jayakumar; B Senthil Kumar. *Int J Pharm Tech*. **2009**, 1(3), 924-935.
[33] PD Mayne. In: Clinical chemistry in diagnosis and treatment, 6th edition, Edward Arnold Publications, London, **1994**; 2-24.
[34] B Isik; R Bayrak; A Akcay. *Mol Cell Biochem*. **2006**, 287, 185-191.
[35] A Karadeniz; A Yildirim; N Simsek; Y Kalkan; F Celebi. *Phytotherapy Res.* **2008**, 22, 1506-1510.
[36] M Ajami; E Shahriar; PTC Hamidreza; H Rouhollah; AE Soltan. *Biol Res*. **2010**, 43, 83-90.
[37] AA Adeneye; JA Olagunju; AS Benebo; SO Elias; AO Adisa; BO Idowu; MO Oyedeji; EO Isioye; OB Braimoh; OO Oladejo; EO Alana. *Int J Appl Res Nat Prod*. **2008**, 1, 6-14.
[38] P Abraham. *Clin Exp Nephrol*. **2005**, 9, 24-30.
[39] MM Ghaisas; VV Navghare; AR Takawale; VS Zope; MA Phanse. *Ars Pharmaceutica*. **2010**, 51(4), 195-206.
[40] NJ Miller; CA Rice-Evans. *Free Radical Res*. **1997**, 26,195-199
[41] AA Adeneye; AS Benebo. *J Ethanopharmacol*. **2008**, 118, 318-323.
[42] R Rastogi; BN Mehrotra. Compendium of indian medicinal plants, CDRI & NISCOM, Lucknow New Delhi, **1970-1979**, 2.