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Research Article

Evaluation of Nephroprotective and antioxidant potential of *Tragia involucrata*

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ABSTRACT

Tragia involucrata (TI) Linn is a shrub belongs to family *Ephorbiaceae* widely distributed in the Indian subcontinent. TI is used for treatment of various diseases including eczema, inflammation, superficial skin infections. Juice from its leaves is commonly used for illnesses including liver and renal conditions in the Asian subcontinent. The aim of this study was to investigate the nephroprotective and antioxidant activities of ethanol extract of TI at two dose levels of 250 and 500 mg/kg B/W on acetaminophen (APAP) induced toxicity in male albino rats. APAP significantly increased levels of serum urea, hemoglobin (Hb), total leukocyte count, packed cell volume,creatinine, , DLC, and mean corpuscular volume, raised body weight, and reduced levels of neutrophils, mean corpuscular Hb content, mean corpuscular hematocrit, granulocytes, uric acid, and platelet Concentration. TI inhibited the hematological effects of APAP. TI significantly increased activities of renal superoxide dismutase, catalase, glutathione, and glutathione peroxidase and decreased malondialdehyde content of APAP-treated rats. Apart from these, histopathological changes also showed the protective nature of the TI extract against APAP induced necrotic damage of renal tissues. In conclusion it was observed that the ethanol extract of AC conferred nephroprotective and antioxidant activities by histopathological and biochemical observations against APAP induced renal damage in rats.

Keywords: *Tragia involucrata*, nephroprotective, antioxidant,Acetaminophen

INTRODUCTION

Acetaminophen (APAP) is well known analgesics as well as antipyretic [1]. Over dose APAP consequentially lead to renal [2-4] and hepatic [5-7] damage. Even if nephrotoxicity is less common than hepatotoxicity in APAP overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury[8-10] and can even lead to fatality in humans and experimental animals. [11-12] Studies are being carried out allover the world in search of molecules involved in the protection of kidney, liver and other vital organs without side effect or less side effect[13-14]. Numerous herbs are traditionally used in various countries in response to drug or toxin induced hepatic and renal disorders. [15]

Tragia involucrata Linn is a shrub belongs to family *Ephorbiaceae* widely distributed in the Indian subcontinent. The "Malaiali" tribes in Western Ghats of India use different parts of *Tragia involucrata* to treat scabies, eczema, inflammation, superficial skin infections as well as wounds [16]. The extract of whole plant used for hypoglycemic activity. [17-18].The root is used for treatment for cold, leg and arm pain, skin eruptions, venereal diseases, blood purification, neuroleptic and fever [19-20] Decoction of the roots was found to be useful in relieving bronchitis and the attendant fever, asthma [21]. Decoction of leaves is taken with the leaves of *Tragia involucrata* and *Aristolochia talaga* to cure scorpion, insect and snake bites. Powder of leaf and root bark is taken with the leaves of *Wrightia tinctoria* and *Tragia involucrata* to make paste and is applied externally to treat skin diseases. [22]

Nevertheless, the nephroprotective effects of this plant extract have not been shown in scientific research work. Taking this into consideration the present study is focused to evaluate the nephroprotective and antioxidant activities of ethanolic extract of *Tragia involucrata* against APAP induced toxicity in rats.

MATERIALS AND METHODS

Plant material

Tragia involucrata leaves were collected from Tirumala hills of Chittoor district, Andhra Pradesh, India and the plant material was

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taxonomically identified and authenticated by the botanist, Voucher specimen (AECBT-07/2007-2008) of this plant has been retained in the Anna Bioresearch foundation, Arunai engineering college, Tiruvannamalai, Tamilnadu, India.

Extraction

The aerial part of *Tragia involucrata* was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60°C [23]. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccators.

Animals

Studies were carried out using Wistar albino male rats (150-200g), obtained from Indian Veterinary Preventive medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 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 Poultry Research Station, Nandhanam, India and fresh water *ad libitum.* All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Acetaminophen induced nephrotoxicity in rats

Animals were randomized and divided into four groups (I – IV) of six animals in each group. Group I served as untreated control and was fed orally with normal saline 5 ml/kg body weight daily for 7days. Group II rats were similarly treated as group I. Groups III and IV animals were treated with 250 mg/kg and 500 mg/kg body weight of the ethanol extract of *Tragia involucrata* for 7 days, respectively. On the 7th day, acetaminophen suspension was given by oral route, in a dose of 750 mg/kg body weight to all rats except the rats in group I.

Hematological study

After 48 h, animals were sacrificed by chloroform anesthesia. Blood samples were collected by cardiac puncher under diethyl ether anesthesia, using 21 gauge (21 G) needles mounted on a 5ml syringe (Hindustan syringes and medical devices ltd, Faridabad, India.) into ethylene diamine tetra-acetic acid (EDTA) – coated sample bottles for analyzed Hematological parameters like full blood count (FBC), hemoglobin, (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet concentration (PLC) and Total leucocytes count (TLC). These parameters were analyzed using automatic hematological system (Sysmex Hematology – Coagulation system, Model MO-1000 I, Trans Asia, Japan).

Sampling and biochemical analysis

Following termination of the experiment on the day 7, the rats were fasted overnight for 14 hours. Blood samples were collected by cardiac puncture with 21G needle mounted on 5 ml syringe (under diethyl ether anesthesia) and centrifuged for 10min at 5000 rpm. The obtained clear sera were stored at -20°C for subsequent measurement of blood urea, creatinine and uric acid 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) levels and antioxidant enzyme levels such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRD) 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 [24]. SOD activity was determined according to the previous report [25] CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method [26]. 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 [27] Glutathione reductase activity was assayed according to the previous reports [28-29]. Protein content in the tissue was determined by the method reported earlier [30] using bovine serum albumin (BSA) as the standard.

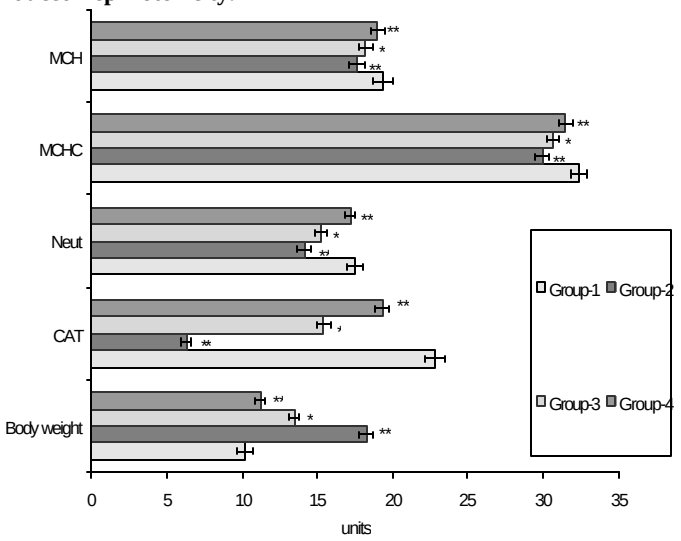
Histopathological examination

Pieces of kidney 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 hematoxylin–eosin. The sections were evaluated for the pathological symptoms of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

RESULTS

Impact of *Tragia involucrata* extract on serum urea, uric acid and creatinine concentrations.In APAP treated group of animals the concentrations of serum urea and creatinine concentrations were considerably increased ($p<0.01$) than the normal animals indicating the severe nephrotoxity(Fig:1).Treating (Group III & IV) with the ethanol extract of *Tragia involucrata* showed significant decrease ($p < 0.05$ & $p < 0.01$) in concentration of serum urea and creatinine compared to APAP treated group. Nevertheless the concentration of uric acid significantly decreased ($p<0.01$) in the APAP treated groups (Group II. Fig 3) than control group. Treatment with ethanol extract of *Tragia involucrata* significantly ($p < 0.05$ & $p < 0.01$) (Group III & IV respectively) increased the uric acid levels, compared to the APAP treated group.

Fig.1. Effect of treatment with ethanol extract of *Tragia involucrata* the renal intracellular CAT activity & blood haemaological parameters (Neutrophil, MCHC & MCH), in rats with acetaminophen (APAP)-induced nephrotoxicity.

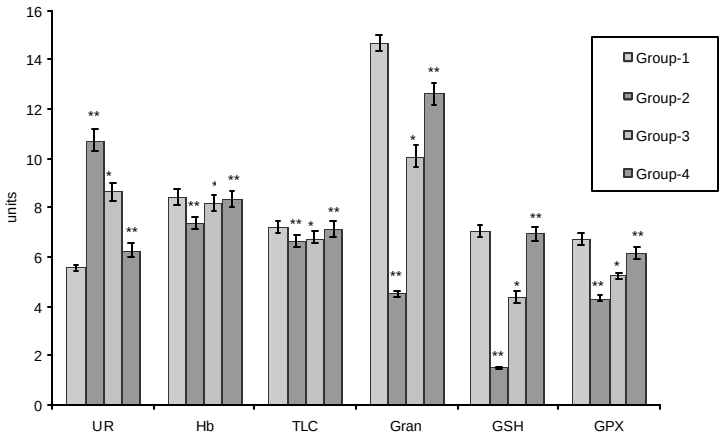


All values are mean ± S.D., (n = 6). **p < 0.01, *p < 0.05 with respect to control. (One way ANOVA followed by Dunnett's t-test.)

Impact of ethanol extract of *Tragia involucrata* on hematological parameters

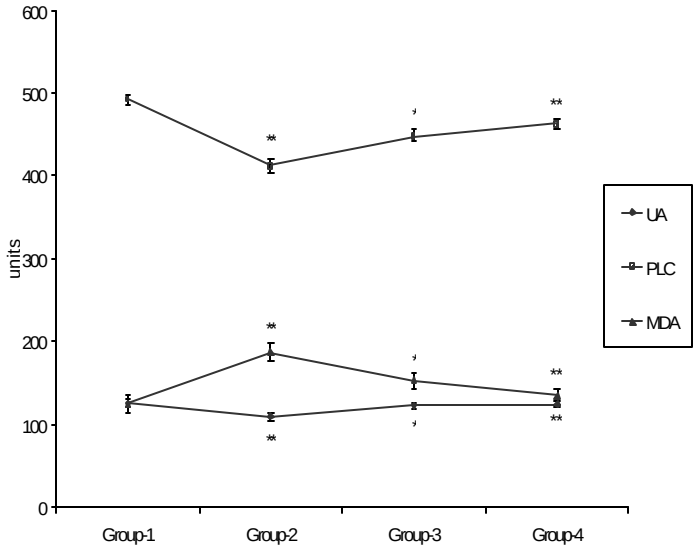
APAP increased (P<0.01) the levels of Hb. PCV and MCV (Fig 2&4) (Group II), consequently caused acetaminophen associated nephropathy. Decrease (Group III & Group IV(p<0.05, p<0.01 respectively) in the Hb, PCV,DLC and MSV levels is achieved by the administration of ethanol extract of *Tragia involucrata* as compared to the APAP induced group (Group II). Moreover, in APAP treated group (Group II), the levels of PLC, MCHC, MCH & lymphocyte are decreased significantly (p<0.01) when compared with normal (Group I) (Fig1,2&3). Administration of *Tragia involucrata* ethanol extract ensures that these levels are retrieved normally, significantly (P<0.05, P<0.01) when compared with Group II.

Fig.2. Effect of treatment with ethanol extract of *Tragia involucrata* the renal intracellular GPX, GSH activity, blood Hematological parameters (Gran,TLC & Hb) and serum urea (UR) levels, in rats with acetaminophen (APAP)-induced nephrotoxicity.



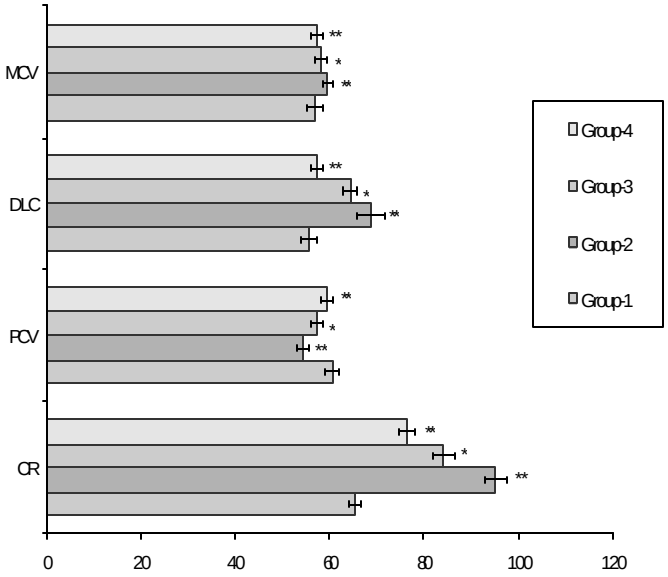
All values are mean ± S.D., (n = 6). **p < 0.01, *p < 0.05 with respect to control. (One way ANOVA followed by Dunnett's t-test.)

Fig. 3. Effect of treatment with ethanol extract of *Tragia involucrata* the renal MDA level, blood hematological parameter (PLC) and serum uric acid levels, in rats with acetaminophen (APAP)-induced nephrotoxicity.



All values are mean ± S.D., (n = 6). **p < 0.01, *p < 0.05 with respect to control (One way ANOVA followed by Dunnett's t-test.)

Fig.4. Effect of treatment with ethanol extract of *Tragia involucrata* the blood hematological parameter (MCV, DLC, PCV) and serum creatinine levels, in rats with acetaminophen (APAP)-induced nephrotoxicity.



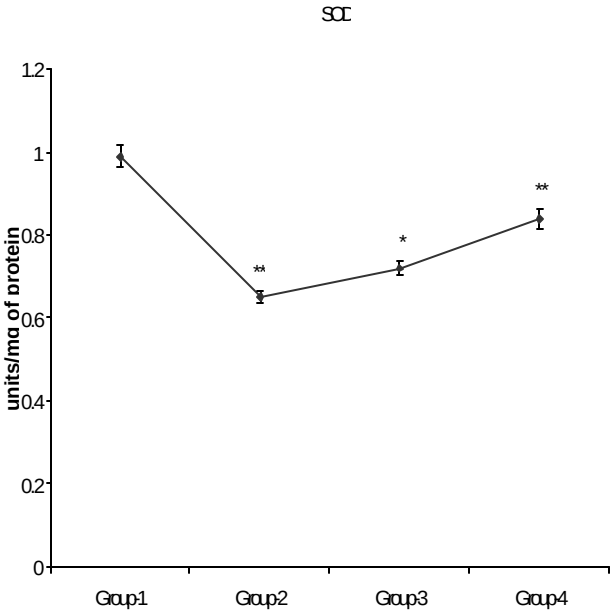
All values are mean ± S.D., (n = 6). **p < 0.01, *p < 0.05 with respect to control. (One way ANOVA followed by Dunnett's t-test.)

Impact of the *Tragia involucrata* extract on kidney antioxidant status

Considerable decrease in the activity of CAT in APAP treated animal group were observed when compared to normal animals (Group I). Treatment with the ethanol extract of *Tragia involucrata* significantly (p < 0.05 & p < 0.01) (Group III & IV) prevented decrease in the level of catalase activity (Fig.1) compared to the APAP induced rat

(Group II). Also GPx activity reduction was observed as result of treatment with APAP was restored by *Tragia involucrata* extract ($p < 0.05$ & $p < 0.01$) (Fig 2) for Group III & IV as compared to the normal group. Renal SOD activity was decreased significantly ($p<0.01$) in the APAP treated (group II) animals compared to normal group. Treatment with the ethanol extract of Tragia *involucrata* (250 & 500 mg/kg body wt) (Group III &IV) significantly ($p<0.05$& $p<0.01$ respectively) elevated the SOD levels as compared to the APAP induced (Group II) animals (Fig.5). The GSH and MDA levels of APAP and extract treated animals are presented in (Fig.2&3). The GSH level reduced significantly ($p < 0.01$) along with increased in MDA concentration in the APAP treated group as compared to the Group I. Anyhow on treatment with *Tragia involucrata* ethanol extract, the GSH level was found to be enhanced significantly ($p<0.05$& $p<0.01$) and the MDA contents were reduced in Group III &IV as compared to the induced group (Group II) (Fig.2).

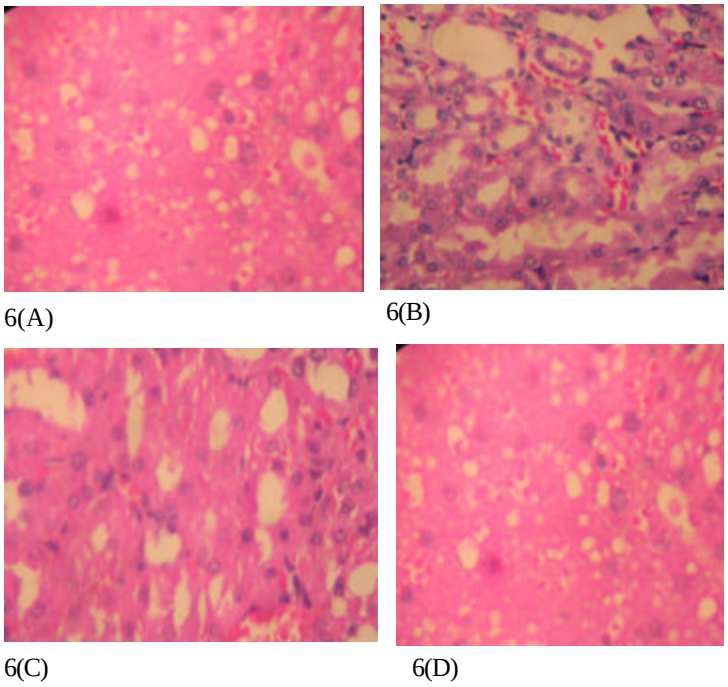
Fig.5. Effect of treatment with ethanol extract of *Tragia involucrata* on renal SOD activity in rats with acetaminophen (APAP)-induced nephrotoxicity.



All values are mean ± S.D., (n = 6). **p < 0.01, *p < 0.05 with respect to control. (One way ANOVA followed by Dunnett's t-test.)

Histopathological Studies

Histological pattern of normal kidney showing normal tubular brush borders and intact glomeruli and Bowman's capsule (Fig.6A) confirms the biochemical results. Severe tubular necrosis and degeneration has shown in the renal tissue (Fig.6B) is the consequential result of APAP. The rats treated with ethanolic extract of *Tragia involucrata* (250mg/kg body weight) showed normal tubular pattern with a mild degree of swelling, necrosis and degranulation (Fig.6C) treatment with the extract (500 mg/kg body weight) ameliorated the toxic manifestations in the kidney (Fig.6D).



6(A) 6(B)

6(C) 6(D)

Fig.6. Nephroprotective effect of *Tragia involucrata*. Histopathological observations (kidney sections stained with Hematoxylin-Eosin, magnification-100x) (A) Normal, (B) Acetaminophen(APAP), (C) Extract 250mg/kg + APAP, (D) Extract 500 mg/kg + APAP.

DISCUSSION

APAP over dose consequentially linked too many metabolic disorders including serum electrolyte, urea and creatinine dearrangements. Increased concentration of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animals and man [31]. The vital function that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoeitic system unique as a target organ [32]. Erythrocytes, leucocytes, and platelets are produced at a turnover rate of about 1 to 3 million per second in a healthy human adult and this value could be altered in certain physiological or pathological states including hemolytic anemia or suppressive inflammation [33]. Drugs like alkylating cytotoxic agents could affect blood formation rate and normal range of hematological parameters [31]. In this study, oral dose of APAP considerably increased Hb, PCV, DLC & MCV levels. Administration ethanol extract of *Tragia involucrata,* these levels is notably decreased compare to the APAP induced group. Whereas the levels of granulocyte, MCH, MCHC and PLC were decreased significantly in the APAP treated group, compared to the normal control group. However after administration of ethanol extract of *Tragia involucrata,* these levels are notably increased compared to the APAP treated group. Nevertheless this study shows that the *Tragia involucrata* extract may contain candidate molecules reversing the hematotoxic effect of acetaminophen, with ensuing improvement of hematopoiesis.

Kidney excretes blood urea nitrogen found in the liver protein which are derived from diet or tissue source. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance [34]. Elevation of urea and creatinine levels in the serum was taken as the index of neph-

rotoxicity[31] [35-36] Creatinine derives from endogenous sources by tissue creatinine breakdown[35]. Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine. In the present study, administration of hepatotoxic and nephrotoxic doses of APAP to rats resulted in development of oxidative stress damage in hepatic and renal tissues. In this study, APAP induced nephrotoxicity showed a considerable (P<0.01) increase in the serum urea and creatinine concentrations in the Group II (APAP induced) rat when compared to the normal group (Group I). In addition, oral administration of ethanol extract of *Tragia involucrata* significantly (P<0.01) decreased in group III & IV when compared to the Group II. Anyhow the level of uric acid is significantly decreased (P<0.01) in the Group II rats when compared to Group I. Oral administration of plant extract significantly (P<0.01) increases the uric acid level in Group I when compared to the APAP induced rats (Group II).

Thus, oxidative stress and lipid peroxidation are early events related to radicals generated during the hepatic metabolism of APAP. Also the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity [12]. Acute APAP overdose increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue [4],[37]Administration of ethanol extract of *Tragia involucrata*, the levels of MDA decreased notably when compared to APAP induced rats.

Superoxide radicals are generated during kidney damage. At the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism [38-39]. This study also demonstrated that acute APAP overdose resulted in a decrease in the SOD, CAT and GST activities, when compared with normal control rats due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. On administration of ethanol extract of *Tragia involucrata*, SOD, CAT and GST reduced activity was increased considerably compared with induced group (P<0.01) (Group II).

Current evidence suggests that intracellular GSH plays an essential role in detoxification of APAP and prevention of APAP-induced toxicity in the liver and kidney[40-42]. Nevertheless microsomal superoxide and hydrogen peroxide production is increased in mice by intake of APAP. The generation of the reactive oxygen species appears as an early event which precedes intracellular GSH depletion and cell damage in APAP hepatotoxicity[43]. Administration of APAP also caused a significant decrease in GSH content. Administration of ethanol extract of *Tragia involucrata*, helped to uplift the GSH depletion induced by APAP.

Biochemical measurements and histopathological changes APAP-induced nephrotoxicity was evidenced coincide with the observations of other investigators[44-45]. The biochemical results were also confirmed by the histological findings which showed preservation of the glomeruli and the surrounding Bowman's capsule and mildly swollen tubules. Other nephroprotective medicinal plants have been reported of inhibiting xenobiotic-induced nephrotoxicity in experimental animal models due to their potent anti-oxidant or free radicals scavenging effects [46]. In addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity[47]. The presence of alkaloids could be the reason of protection offered by the extract [48]. The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. The findings suggest the potential use of the ethanol extract of *Tragia involucrata* 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.

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