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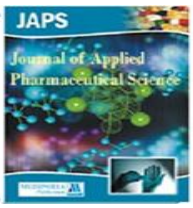

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Evaluation of phytoconstituents, nephro-protective and antioxidant activities of *Clitoria ternatea*

K.Sarumathy, M.S.Dhana Rajan, T.Vijay,J.Jayakanthi

K.Sarumathy , J.Jayakanthi
[a] *Research and development centre, Bharathiar university, Coimbatore, India.*
[b]*P.G & Research Dept. of Biochemisry, DKM College for Women, Vellore, India*

M.S.Dhana Rajan
P.G & Reseach Dept. of Biochemistry Jaya college of Arts & Science, Tiruninravur, Chenna, India.

T.Vijay
[a] *Research and development centre, Bharathiar university, Coimbatore, India.*
[b]*P.G & Research Dept. of Biochemisry, KMG College of Arts & Science, Gudiyattam Vellore dist., India*

***For Correspondence**:*
K.Sarumathy
PG & Research Dept. of Biochemistry D.K.M College for Women Vellore-632 001, India.
Mail: vcharujee@gmail.com

ABSTRACT

Clitoria ternatea(CT), is an herbaceous medicinal plant used to treat, liver problems India. Acetaminophen is a commonly used analgesic and antipyretic agent which, at high doses, causes liver and kidney necrosis in man and animals. The aim of the present study is to evaluate phytoconstituents and investigate the nephroprotective & antioxidant activities of the ethanol extract of *Clitoria ternatea* on acetaminophen induced toxicity in rats. Phytoconstituents like 1H-Cycloprop[e]azulene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1aà,7à,7aá,7bà)]- [Synonyms: Varidiflorene], Pterocarpin, 6H-Benzofuro[3,2-c][1]benzopyran, 6a,11a-dihydro-3,9-dimethoxy-, (6aR-cis)- [Synonyms: Homopterocarpin], Isoparvifuran, Hexadecanoic acid, ethyl ester, Myo-Inositol, 4-C-methyl-, 1,2,3,5-Cyclohexanetetrol, (1à,2á,3à,5á)-, Propane, 1,1-diethoxy- were identified from ethanol extract of *Clitoria ternatea* by using a gas chromatograph-mass spectrograph (GC MS). Biochemical studies show that there is an increase in the levels of serum urea and creatinine along with an increase in the body weight and reduction in the levels of uric acid in acetaminophen induced groups. These values are retrieved significantly by treatment with *Clitoria ternatea* extracts at two different doses. The antioxidant studies reveal that the levels of renal SOD, CAT, GSH and GPx in the APAP treated animals are increased significantly along with a reduced MDA content in ethanol extract of *Clitoria ternatea* treated groups. Apart from these, histopathological changes also reveal the protective nature of the *Clitoria ternatea* extract against acetaminophen induced necrotic damage of renal tissues. In conclusion, these data suggest that the ethanol extract of *Clitoria ternatea* can prevent renal damage from APAP induced nephrotoxicity in rats and it is likely to be mediated through active phytoconstituents and its antioxidant activities.

Key words: *Clitoria ternatea*, antioxidant, acetaminophen, nephroprotective.

INTRODUCTION

Acetaminophen (APAP) is a widely used analgesic and antipyretic drug that is safely employed for a wide range of treatments (Yapar *et al*., 2007), Overdose of APAP in human is fairly common and is often associated with hepatic (Yank *et al*., 1999, Flanagin *et al*., 2002, Godlee employed for a wide range of treatments (Yapar *et al*., 2007), Overdose of APAP in human is *et al*., 1999) and renal damage (Pitkin *et al*., 1999). Although 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 (Carpenter *et al*., 1981, Jones *et al*., 1993, Eguia *et al*., 1977) and can even lead to death in humans and experimental animals (Ray *et al*., 1996, Webster *et al*., 1996). Studies are going on throughout the world for the search of protective 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 (Montilla *et al*., 2005, Mansour *al*., 2006). A number of herbs are traditionally used in different countries in response to drug or toxin induced hepatic and renal disorders (El-Beshbishy *et al*., 2005). *Clitoria ternatea* Linn (Family Papilionaceae), is commonly known as “Butterfly pea”. It is a tropical plant found in India, the root and rootbarks are used in Ascathartic, diuretics and has laxative

effects (Chopra RN et al., 1956). The flowers and leaves of CT are used to make collyrium, leaves are also used in Madagascar to relieve joint pain, and hepatopathy, the seeds of CT have laxative effects, and are cathartic, the root juice of CT used in the treatment of Chronicbronchitis and the leaves are useful in otalgia and hepatopathy, whereas seeds are cathartic (Anonymous,1995). It contains antifungal proteins, and has been shown to be homologous to plant defensins (Osborn et al.,1995 Rai et al, 2001). In recent years GC-MS have been applied to unambiguously identify the structures of different phytoconstituents in plant extracts and biological samples with great success (Prasain *et al*., 2004; DE Rijke *et al*., 2006). GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. (Anjali *et al*., 2009). The present study is, therefore, aimed to evaluate the phytoconstituents and nephroprotective and antioxidant activities of ethanolic extract of *Clitoria ternatea* (EECT) against acetaminophen induced toxicity.

MATERIALS AND METHODS

Plant material

Aerial part of *Clitoria ternatea* Linn (Family Papilionaceae), was collected from Tirunelveli district, Tamil Nadu, India in the month of March. The plant material was taxonomically identified and authenticated by V Chelladurai (Research Officer), Botany (CCRAS) Government of India. Voucher specimen (DKMC-05/2010-2011) has been retained in DKM College for women ,Vellore, Tamilnadu, India.

Gas chromatography-mass spectrometer

The analytical column of HP-1MS (Agilant 6890/ Hewlett-Packard 5975) capillary column was used. Helium was used as the carrier gas at a flow rate of 1 mL/min. The temperature was programmed at 80ºC for 5 min and then increased to 300ºC at the rate of 15ºC/min. The temperatures of injector and EI detector (70eV) were maintained at 280ºC and 300ºC, respectively.

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^{o}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 the experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Extraction

The aerial part of *Clitoria ternatea* 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^{o}C (Chattopadhyay, 2003). The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccator.

GC-MS analysis of ethanol extract of *CLITORIA TERNATEA* for identification of chemical composition

The identification of chemical composition of ethanol extract of *Clitoria ternatea* was performed using a gas chromatograph-mass spectrograph (GC-MS) (Agilant 6890/Hewlett-Packard 5975) fitted with electron impact (EI) mode. 2.0 µL of the ethanol extract of *Clitoria ternatea* was injected with a Hamilton syringe to the GC-MS manually for total ion chromatographic analysis in split mode. In quantitative analysis, selected ion monitoring (SIM) mode was employed during the GC/MS analysis. SIM plot of the ion current resulting from very small mass range with only compounds of the selected mass were detected and plotted.

Experimental treatments

Animals were divided into four groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II treated with a single dose of acetaminophen (APAP) of 750mg/kg body weight was kept as toxin control. Group III and IV were treated with ethanol extract of *Clitoria ternatea* at two different doses of 250 and 500 mg/kg body wt respectively plus APAP. The extract was administered by oral gavages 1 h before APAP administration (Deepak *et al*., 2007).

Hematological study

After 48 h, the animals were sacrificed by chloroform anaesthesia. 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 concen-tration (PLC) and total leucocyte 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 14, 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^{o}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 −8oC. Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000rpm for 10 min at 4oC. 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 (26). SOD activity was determined according to the previous report (Rai *et al*., 2006). CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method (Bergmeyer *et al*., 1974). 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 (Hafemann *et al*., 1974). Glutathione reductase activity was assayed according to the previous reports (Carlberg *et al*., 1975, Mohandas *et al*., 1984). Protein content in the tissue was determined by the method reported earlier (Lowry *et al*., 1951) using bovine serum albumin (BSA) as the standard.

Histopathological analysis

A portion of the kidney tissue was immediately kept in 10% formalin to fix the tissue after sacrifice. The tissues were washed in running tap water, dehydrated in the descending grades of isopropanol and finally cleared in xylene. The tissues were then embedded in molten paraffin wax. Section 10µm tthickness were cut and stained with haematoxylin and eosin. The sections were then viewed under light microscope for histopthological changes.

RESULTS

Phytochemical analysis

The ethanol extract of *Clitoria ternatea* was a complex mixture of many constituents, and seven compounds were identified in this plant by GC-mass spectroscopy for the first time. Phytoconstituents such as n-hexadecanoic acid (48.77), 1-butanol, 3-methyl-acetate (30.27), propane, 1,1,3-triethoxy-(3.92), Z, Z, Z-1, 4, 6, 9-nonadecatetraene (4.60), undecanoic acid (2.80), 3-trifluoroacetoxy pentadecane (3.59), and 4-ethyl - 5-octyl- 2, 2- bis (trifluoromethyl) – cis 1, 3 - dioxalone - (6.05) were identified in the ethanol extract of *Clitoria ternatea* by relating to the corresponding peak area through coupled GC-mass spectroscopy (GC-MS) (table 1; fig. 1).

Effect of *Clitoria ternatea* extract on serum urea, uric acid and creatinine concentrations

Serum urea and creatinine concentrations were significantly increased ($p<0.01$) in the APAP treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity (figs. 2-4). Treatment with the ethanol extract of *Clitoria ternatea* showed significant ($p<0.05$ and $p<0.01$) (Group III and IV) decrease in concentrations of serum urea and creatinine compared to the APAP treated group. However, the levels of uric acid (UA) significantly decreased ($p<0.01$) in the APAP treated groups (Group II, fig. 3), when compared to the control group. Treatment with ethanol extract of *Clitoria ternatea* significantly ($p<0.05$ and $p<0.01$) (Group III and IV respectively) increased the uric acid levels, compared to the APAP treated group.

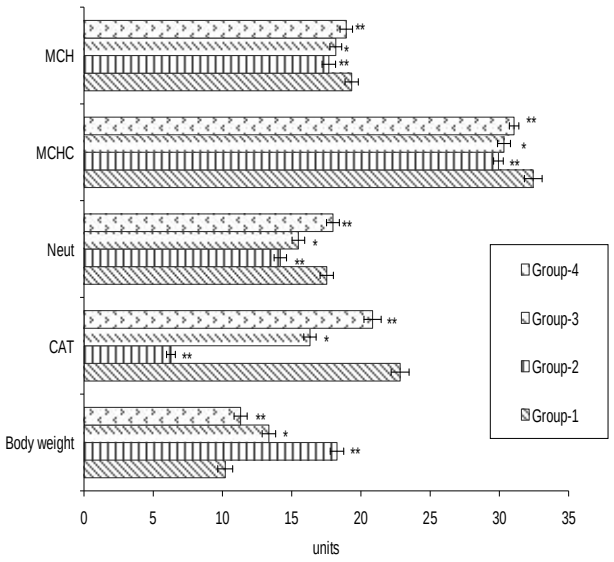

Fig. 1: Effect of *Clitoria ternatea* extract on the alterations induced by administration of 750 mg/kg/day acetaminophen (APAP) to rats for 14days in the in the renal intracellular CAT activity, blood Haemaological parameters (Neutrophil,MCHC &MCH) and body weight changes. All values are mean ± S.D. (n = 6). **p<0.01, *p<0.05 with respect to control. (One way ANOVA followed by Dunnett's t-test).

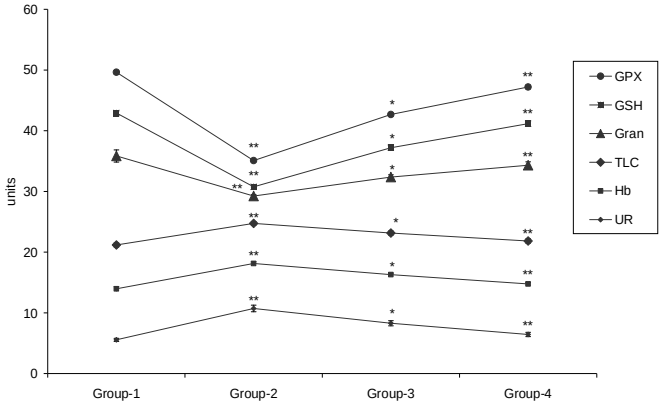

Fig. 2: Effect of extract *Clitoria ternatea* on the alterations induced by administration of 750 mg/kg /day acetaminophen (APAP) to rats for 14days in the in the renal renal intracellular GPX, GSH activity, blood Haemaological parameters (Gran, TLC and Hb) and serum Urea levels. All values are mean ± S.D. (n = 6). **p<0.01, *p<0.05 with respect to control (One way ANOVA followed by Dunnett's t-test).

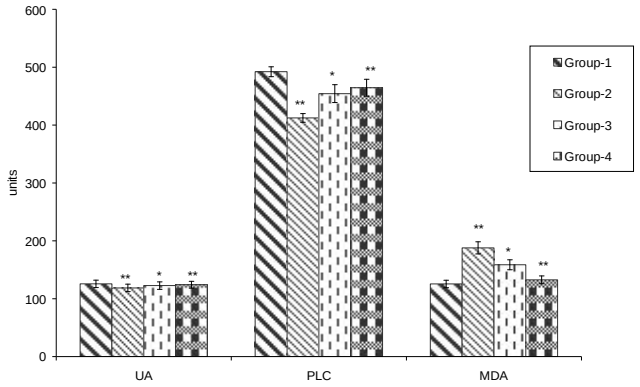

Fig. 3: Effect of *Clitoria ternatea* extract on the alterations induced by administration of 750 mg/kg/day acetaminophen (APAP) to rats for 14 days in the in the serum uricacid level, blood Haemaological parameter (PLC) and renal MDA level. All values are mean ± S.D. (n = 6). **p<0.01, *p<0.05 with respect to control (One way ANOVA followed by Dunnett's t-test).

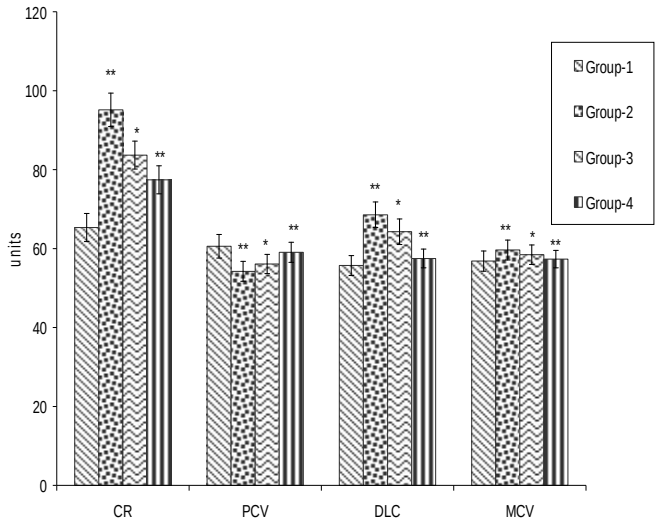

Fig. 4: Effect of *Clitoria ternatea* extract on the alterations induced by administration of 750 mg/kg/day acetaminophen (APAP) to rats for 14 days in the serum creatinine level and blood Haematological parameter (PCV, DLC, MCV) All values are mean ± S.D. (n = 6). **p<0.01, *p<0.05 with respect to control (One way ANOVA followed by Dunnett's t-tes.t).

Effect of ethanol extract of *Clitoria ternatea* on hematological parameters

APAP caused a significant (P<0.01) decrease in the Hb and PCV levels (figs. 2 and 4) resulting in acetaminophen associated nephropathy. Administration of ethanolic extract of *Clitoria ternatea* significantly (p<0.01) increased the Hb and PCV (figs. 2 and 4) values recorded for APAP hematotoxicity and also caused a significant (p<0.01) increase in DLC and MCV levels in APAP treated animals (Group II, fig. 4). However, the administration of ethanol extract of *Clitoria ternatea* (Group III and IV) reversed the significant (p<0.01) increase in DLC and MCV levels. Further, in the APAP treated group (Group II), the levels of PLC, MCHC and lymphocyte are decreased significantly (p<0.01) when compared with normal (Group I) (fig. 1). By the administration of ethanol extract of *Clitoria ternatea*, these levels are retrieved normally. Also the levels of PLC, APAP treated animals are decreased significantly (p<0.01) when compared with Group I, even though by the administration of ethanol extract of *Clitoria ternatea* (250 mg and 500mg), the PLC level was increased significantly (p<0.05 and p<0.01 respectively) (fig. 3).

Effect of the *Clitoria ternatea* extract on kidney antioxidant status

The activity of CAT in the APAP treated group was significantly (p<0.01) decreased when compared to the normal animals (Group I). Treatment with the ethanol extract of *Clitoria ternatea* significantly (p<0.05 and p<0.01) (Group III and IV) prevented the decrease in the level of catalase activity (fig. 1) compared to the APAP induced rat (Group II). Likewise, the decreased GPx activity as a result of the treatment with APAP was also restored by the ethanol extract of *Clitoria ternatea* (p<0.05 and p<0.01) (fig. 2) for Group III and IV as compared to the normal group. Renal SOD activity was decreased significantly (p<0.01) in the APAP treated (group II) animals as compared to the normal group. Treatment with the extract (250 and 500 mg/kg body wt) (Group III and IV) significantly (p<0.05 and p<0.01 respectively) elevated the SOD levels as compared to the APAP induced (Group II) animals (fig. 5).

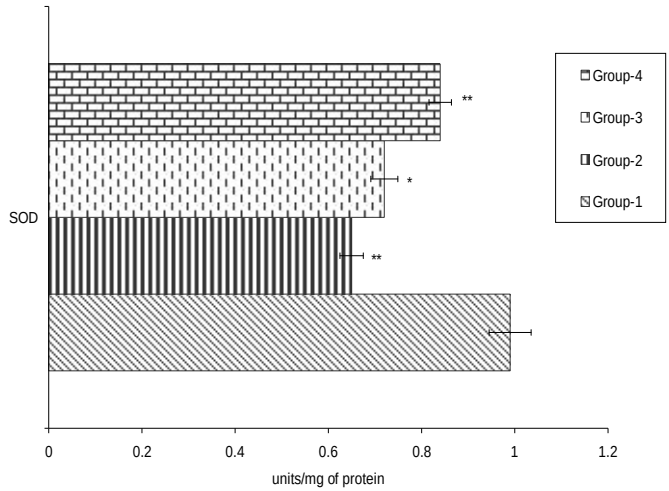

Fig. 5: Effect of *Clitoria ternatea* on the alterations induced by administration of 750 mg/kg/day aceta-minophen (APAP) to rats for 14 days in the renal intracellular SOD levels. All values are mean ± S.D. (n = 6). **p<0.01, *p<0.05 with respect to control (One way ANOVA followed by Dunnett's t-test).

The GSH and MDA levels of APAP and extract treated animals are presented in (figs. 2 and 3). The GSH level reduced significantly (p<0.01) along with an increase in MDA concentration in the APAP treated group as compared to the Group I. However, on treatment with ethanol extract of *Clitoria ternatea*, the GSH level was found to be enhanced significantly (p<0.05 and p<0.01) and the MDA contents were reduced in Group III and IV as compared to the induced group (Group II) (fig. 3).

Histopathological studies

The biochemical results were also confirmed by the histological pattern of normal kidney showing normal tubular

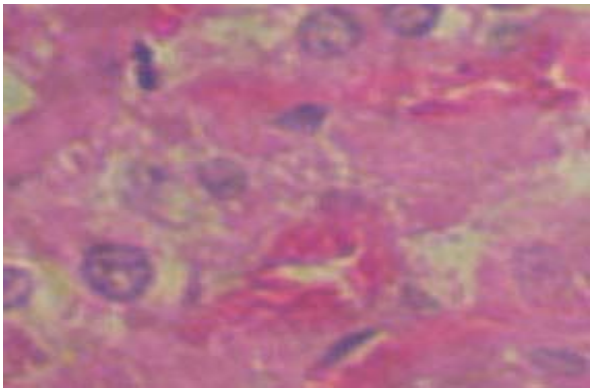

Fig. 6a: A representative section of a normal kidney showing normal tubular brushborders and intact glomeruli and Bowman's capsule (×100 magnification, hematoxylin and eosin stain).

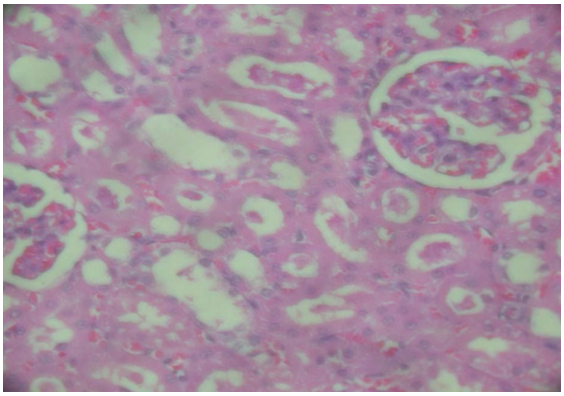

Fig. 6b: Light microscopy of renal tissue in acetaminophen treated rat. Sever tubular necrosis and degeneration are shown.

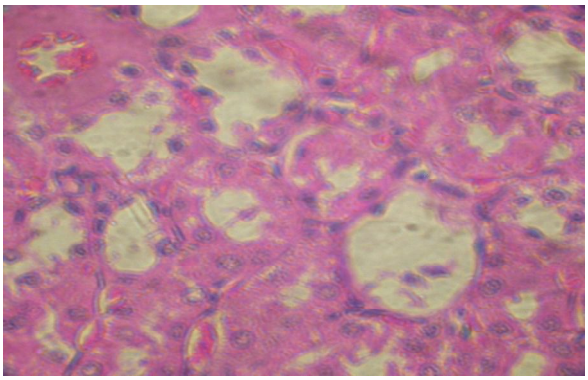

Fig. 6c: Light microscopy of renal tissue in 250 mg *Clitoria ternatea* extract treated showed mild degree of necrosis and degeneration are shown.

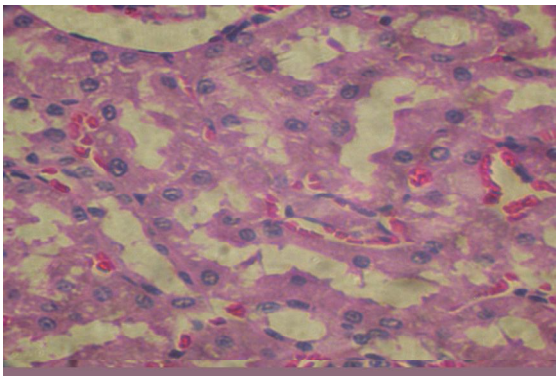

Fig. 6d: Representative of oral 500 mg/kg/day of CT-treated acetaminophen induced nephrotoxic rat kidney showing focal renal glomerulus mild lymphocytic infiltration and diffuse mildly swollen tubules (Hematoxylin and Eosin stain 100X).

brush borders and intact glomeruli and Bowman's capsule (fig. 6a). Severe tubular necrosis and degeneration is shown in the renal tissue on treatment with acetaminophen (fig. 6b). The rats treated with ethanol extract of *Clitoria ternatea* (250mg/kg body weight) showed normal tubular pattern with a mild degree of swelling, necrosis and degranulation (fig. 6c). Treatment with the ethanol extract of *Clitoria ternatea* (500 mg/kg body weight) ameliorated the toxic manifestations in the kidney (fig. 6d).

DISCUSSION

The GC device is generally a reliable analytical instrument. The GC instrument is effective in separating compounds into their various components. The MS instrument provides specific results but produces uncertain qualitative results. When an analyst uses the GC instrument to separate compounds before analysis with an MS instrument, a complementary relationship exists. The technician has to access both the retention times and mass spectral data. Many scientists consider GC/MS analysis as a tool for conclusive proof of identity. In the present study, phytoconstituents like 1H-Cycloprop[e]azulene, 1a,2,3,5, 6, 7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,7à,7aá,7bà)]- [Synonyms: Varidiflorene], Pterocarpin, 6H-Benzofuro[3,2-c][1]benzopyran, 6a,11a-dihydro-3,9-dimethoxy-, (6aR-cis)- [Synonyms: Homopterocarpin], Isoparvifuran, Hexadecanoic acid, ethyl ester, Myo-Inositol, 4-C-methyl-, 1,2,3,5-Cyclohexanetetrol, (1à,2á,3à,5á)-, Propane, 1,1-diethoxy- were identified from ethanol extract of *Clitoria ternatea* by using a gas chromatograph-mass spectrograph (GC MS). This identification was done by comparison of their mass spectra on both columns with phytochemical and ethnobotanical databases libraries or with mass spectra from the literature (Adams, 2001; Jennings and Shibamoto, 1980) and home-made library. Most of the compounds belong to the group of antioxidant agents (Yoshida and Nihi, 2003). In recent years, the antimicrobial activity of n-hexadecanoic acid was discussed by (Woolford *et al*., 1975; Dawson, *et al*., 2002; Lee *et al*., 2002; Bergsson *et al*., 2002). Based on the literature data all these compounds could effective contribute to the biological activities of *Clitoria ternatea.*

Acetaminophen overdose is often linked to 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 (Rai *et al*., 2006). The reason behind acetaminophen toxicology is the CYP-mediated conversion of acetaminophen to a highly reactive quinone imine, *N*-acetyl-*p*-benzoquinone imine. The fundamental role of NAPQI in the toxicity of acetaminophen has been supported by many subsequent studies (Corcoran *et al*., 1980, Dahlin *et al*., 1982, Holme *et al*., 1984, Dahlin *et al*., 1984, Streeter *et al*., 1984, Lowry *et al*., 1951). Blood urea nitrogen is found in the liver protein that is derived from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance (Mayne *et al*., 1994). Elevation of urea and creatinine levels in the serum was taken as the index of nephrotoxicity (Anwar *et al*., 1999, Bennit *et al*., 1982, Ali *et al*., 2001). Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown (Mayne *et al*., 1994). Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine. In the present study, administration of APAP to rats resulted in nephrotoxic and development of oxidative stress damage in renal tissues. In this study, APAP induced nephrotoxicity showed a significant ($p<0.01$) increase in the serum urea and creatinine concentrations in Group II (APAP induced) rats when compared to the normal group (Group I). Moreover, oral administration of ethanol extract of *Clitoria ternatea* significantly ($p<0.01$) decreased in Group III and IV when compared to the Group II. However 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 III and IV respectively, 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 (Somani *et al*., 2000). Previous studies have clearly demonstrated that acute APAP overdose increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue (Abdel-Zaher *et al*., 2007, Ghosh *et al*., 2007). However, in the APAP treated animals, the MDA levels are increased significantly, when compared to normal control rats. On administration of ethanol extract of *Clitoria ternatea* , the levels of MDA were decreased significantly when compared to APAP induced rats.

During kidney injury, superoxide radicals are generated 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 (Pande *et al*., 2002, Linares *et al*., 2006). The present study also demonstrated that acute APAP overdose resulted in a decrease in the SOD, CAT and GST activities, when compared with normal control rats. It is due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. When rat was treated with the ethanol extract of *Clitoria ternatea* the reduction of SOD, CAT and GST activity was increased significantly when 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 (Newton *et al*., 1996, Richie *et al*., 1992, Nelson *et al*., 1990). However, APAP was found to increase the microsomal superoxide and hydrogen peroxide production in mice. The generation of the reactive oxygen species appears as an early event which precedes intracellular GSH depletion and cell damage in APAP hepatotoxicity (Manov *et al*., 2003). APAP administration also caused a significant decrease in GSH content. Administration of ethanol extract of *Clitoria ternatea* helped to uplift the GSH depletion induced by APAP.

APAP-induced nephrotoxicity was evidenced by biochemical measurements and histopathological changes that coincide with the observations of other investigators (Gardner *et al*., 2002, Newton *et al*., 1983, Trumper *et al*., 1998). 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

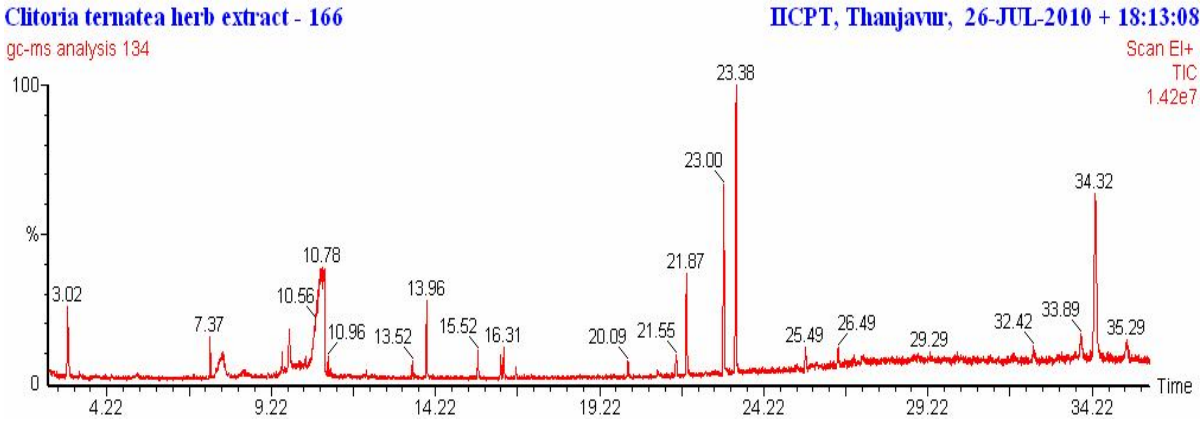

Fig. 7: GC-MS study of *Clitoria ternatea* shows various peak indicates the different chemical compounds.

Table 1 : Activity of Components identified in *Clitoria ternatea* herb extract- *166* [GC MS study].

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1.	3.02	Propane, 1,1-diethoxy-	$C_7H_{16}O_2$	132	4.73	Ether compound	No activity reported
2.	7.37	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	$C_{15}H_{24}$	204	1.18	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
3.	9.77	1,2,3,5-Cyclohexanetetrol, (1à,2á,3à,5á)-	$C_6H_{12}O_4$	148	3.55	Poly Hydroxy compound	Antioxidant Antimicrobial Anti-inflammatory
4.	10.78	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194	31.07	Inositol compound	Antidiabetic Antimicrobial
5.	13.96	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.66	Fatty acid ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
6.	15.52	Phytol	$C_{20}H_{40}O$	296	1.18	Diterpene	Antimicrobial Antiinflammatory Anticancer Diuretic-
7.	16.22	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	0.89	Linoleic acid ester	Antiinflammatory, Hypocholesterolemic, Cancer preventive Hepatoprotective Nematicide, Insectifuge Antihistaminic, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
8.	16.31	7,11-Hexadecadienal	$C_{16}H_{28}O$	236	1.18	Aldehyde compound	Antimicrobial
9.	16.67	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.59	Fatty acid ester	No activity reported
10.	21.87	Isoparvifuran	$C_{16}H_{14}O_3$	254	5.33	Aromatic compound	No activity reported
11.	23.00	6H-Benzofuro[3,2-c][1]benzopyran, 6a,11a-dihydro-3,9-dimethoxy-, (6aR-cis)- [Synonyms: Homopterocarpin]	$C_{17}H_{16}O_4$	284	10.06	Aromatic compound	Antimicrobial
12.	23.38	Pterocarpin	$C_{17}H_{14}O_5$	298	15.68	Ether compound	Antimicrobial
13.	34.32	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,7à,7aá,7bà)]- [Synonyms: Varidiflorene]	$C_{15}H_{24}$	204	21.89	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Antiinflammatory Sedative, Fungicide

xenobiotic-induced nephrotoxicity in experimental animal models due to their potent anti-oxidant or free radicals scavenging effects (Devipriya *et al*., 1999, Annie *et al*., 2005). In addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity (Kumaran *et al*., 2007). The protection offered by the extract could have been due to the presence of flavonoids and alkaloids (Donsky *et al*., 2007, Lucia *et al*., 2007).

CONCULSION

The present study shows that the administration of ethanol extract of CT has Nephroprotective potential against APAP-induced Nephrotoxicity. It provides experimental evidence that CT augmented the myocardial antioxidant enzymes level, preserved histoarchitecture and improved cardiac performance following APAP administration. This Nephroprotective activity of CT might be due to the synergetic effect of chemical compounds present in them making them good sources for the production of a Nephroprotective herbal medicine. The identification of molecules with Nephroprotective potential from this ethanol extract of CT may provide new directions for identification of Nephroprotectives, which could be given concomitantly during APAP treatment.

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