Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 5 (3): 266-273, 2011
ISSN 1819-3455 / DOI: 10.3923/rjmp.2011.266.273

Acute Toxicity and Hepatoprotective Effect of Methanolic Extract of *Tephrosia calophylla*

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ABSTRACT

Carbon tetrachloride (CCl_4) is a known chemical with high incidence hepatotoxicity. The objective of this study was to investigate the acute toxicity and hepatoprotective activity of methanol extract of root of *Tephrosia calophylla* against CCl_4 induced hepatotoxicity. Animals were pretreated with the methanol extract of *Tephrosia calophylla* (150 and 300 mg kg^{-1} b.wt.) for 14 days and then challenged with CCl_4 (1.5 mL kg^{-1} b.wt.) in olive oil (1:1, v/v) on 14th day. Serum marker enzymes (ALP, SGOT, SGPT, total protein, albumin and total bilirubin) were estimated in all the study groups. For acute toxicity study it was found that the LD_{50} value of methanol extract of *Tephrosia calophylla* was 1000 mg. The results of biochemical parameters revealed the elevation of biochemical markers like SGPT, SGOT, ALP and bilirubin in toxicant treated group indicating that CCl_4 induces damage to the liver. Pretreatment with methanolic extract of *Tephrosia calophylla* significantly reduced the elevated levels of SGPT, ALP and Bilirubin, but not SGOT. Low level of total protein and albumin in toxicant treated group and the significant elevation in methanolic extract of *Tephrosia calophylla* treated group shows protection of against in CCl_4 induced liver injury. The extract of *Tephrosia calophylla* treated rats when compared with standard Liv 52 group and extract control group there was no significant different in biochemical parameters.

Key words: Herbal drug, carbon tetrachloride, liver injury, liver marker enzyme

INTRODUCTION

Liver is major organ involved in maintenance of human homeostasis mechanism through metabolic pathway. Various chemical and toxins were affecting the liver during this process. So, identification of hepatoprotective molecule from natural source is very important in this modern life. In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief (Arulkumaran *et al*., 2009). Exposure to various organic compounds including a

number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as Reactive Oxygen Species (ROS). Carbon tetrachloride (CCl_4) is a well-known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals (Manna *et al*., 2006). Many Indian medicinal plants have also been considered to protect health, longevity, intelligence, immunosurveillance and body resistance against different infections and diseases (Manna *et al*., 2006). Many research carried out by using herbal drugs in CCl_4 induced liver injury like *Tephrosia* purpurea (Farghali *et al*., 2000), *Terminalia arjuna* (Manna *et al*., 2006), *Prostechea michuacana* (Gutiérrez and Solis, 2009), *Ficus carica* (Venkatesh *et al*., 2007) etc.

Tephrosia (Leguminosae or Papilinoideae) is a large tropical and sub tropical genus estimated to contain 300 species (Willis, 1973). *Tephrosia calophylla* is perennial undershrub found widely in Talakona forest of Andhra Pradesh, south india (Thammanna *et al*., 1994). The genus tephrosia is known to contain a wide variety of flavonoids (Dewick, 1993). The compound tephlostan, 7-O-methylglabranin and kaempferol-3-O-β-D-glucopyranoside were isolated and characterized from the whole plant of *Tephrosia calophylla*. As the *Tephrosia* genus represents potential source for flavonoids and various biological activities. According to Ayurvedha the plant is useful as an anthelmentic, anti-pyretic and as well as an alexiteric drug. It is also active against leprosy, ulcers and used as alternative cures for diseases of the liver, spleen, heart and blood. According to the Unani system of medicine the root is diuretic, allays, thirst, enriches blood, cures diarrhea and is useful in bronchitis, inflammation, boils and pimples. Leaves are tonic to intestine and a promising appetizer (Adinarayana *et al*., 2009). So, the present investigation was made to study the toxicity profile and hepatoprotective effect of *Tephrosia calophylla* against CCl_4 induced hepatotoxicity.

MATERIALS AND METHODS

Plant material: Fresh roots of *Tephrosia calophylla* were collected from Talakona forest from Andhra Pradesh and identified by Dr. Madhava Chetty, Departement of Botany, Sri Venkateswara University Tirupati the study was conducted in 2010. The shade dried plant materials were powdered and extracted with methanol in soxhlet apparatus. The methanol extract was concentrated in rotary evaporator at a temperature not more than 50°C. The concentrated crude extract was lyophilized in to powder and used for this study.

Animals: Male Wister rats (150-175 g) were procured from King Institute, Chennai, Tamilnadu, India and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature 25±2°C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining Institutional Animal Ethical Committee clearance (Protocol No. 1220/a/08/CPCSEA/ANCP/05).

Acute toxicity studies: Acute Oral Toxicity (AOT) of *Tephrosia calophylla* was determined using Swiss albino mice. The animals were fasted for 3 h prior to the experiment and were administered with single dose of methanolic extract of *Tephrosia calophylla* dissolved in 5% gum acacia (doses ranges from 100-5000 mg kg^{-1} at various dose levels) and observed for mortality up to 48 h (short term toxicity). Based on the short-term toxicity, the dose of next animal was determined as per OECD guideline 425. The LD_{50} of the tes extract was calculated using Graphical method.

Evaluation of hepatoprotective activity: Seven groups of animals containing six each were used for the study. The animals from group I served as the control and received the vehicle saline at a dose of 1 mL/kg/day, p.o. for 14 days. Groups III-VII received Liv 52 and the methanol extract of *Tephrosia calophylla* at a dose of 250 and 500 mg/kg/day, p.o. for 14 days, respectively. Group III-V was treated with the CCL_4 in the dose of 1.5 mL kg^{-1}, p.o. on day 14 days. After 36 h of CCl_4 administration all the animals were killed under chloroform anesthesia. The blood samples were collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 min and serum was collected. The separated serum was analyzed to assess various biochemical markers like Serum Glutamic Pyruvate Transaminase (SGPT) (Reitman and Frankel, 1957), Serum Glutamic Oxaloacetate Transaminase (SGOT) (Reitman and Frankel, 1957), Alkaline Phosphatase (ALP) (Kind and King, 1954), total bilirubin (Mallay and Evelyn, 1937) and total protein.

Statistical analysis: All values were expressed as Mean±SEM. Statistical analysis was performed with one way Analysis of Variance (ANOVA) followed by Dunnett's t-test. The p values <0.05 were considered to be statistically significant when compared to CCl_4 group.

Histopathology: After draining the blood, the abdomen of each animal was cut opened and the liver samples were excised, washed with ice cold saline and processed separately for histopathological observation. The ratio of wet liver weight was calculated. The livers were examined grossly, were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 μm thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin (Galigher and Kozloff, 1971). The sections were examined microscopically for histopathological changes.

RESULTS AND DISCUSSION

Acute toxicity study: By graphical method it was found that the LD_{50} value of methanol extract of *Tephrosia calophylla* was 1000 mg, the results were present in Table 1.

Hepatoprotective activity

Biochemical parameters: The results of biochemical parameters revealed the elevation of biochemical markers like SGPT, SGOT, ALP and bilirubin in toxicant treated group indicating that CCl_4 induces damage to the liver. Pretreatment with methanolic extract of *Tephrosia calophylla* (150 and 300 mg kg^{-1}) significantly reduced ($p<0.001$) the elevated levels of SGPT, ALP and Bilirubin, but not SGOT. Low level of total protein and albumin in toxicant treated group and the significant elevation in methanolic extract of *Tephrosia calophylla* treated group shows protection of *Tephrosia calophylla* extract treatment in CCL_4 induced liver injury. The extract of *Tephrosia calophylla* treated rats when compared with standard Liv 52 group and extract control group there was no significant different in biochemical parameters (Fig. 1a-f).

Table 1: Acute toxicity study of *Tephrosia calophylla*

Dose	Log dose	Dead/total	% of dead	Corrected %	Probit
100	2.000	1/10	10	10.0	3.72
500	2.698	3/10	30	30.0	4.48
1000	3.000	5/10	50	50.0	5.00
3000	3.400	8/10	80	80.0	5.84
5000	3.698	10/10	100	97.5	6.96

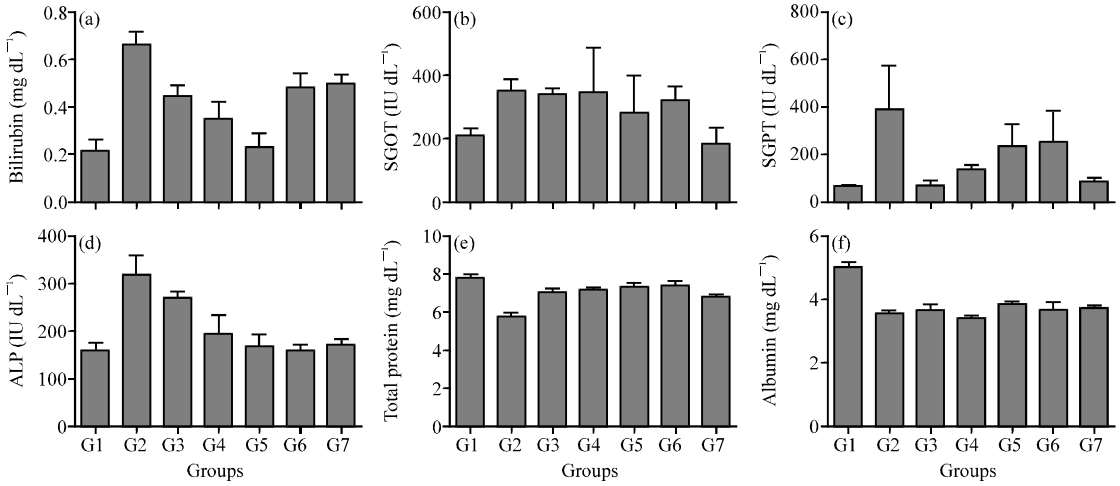

Fig. 1: The serum level of (a) total bilirubin, (b) SGOT, (c) SGPT, (d) ALP, (e) total protein and (f) albumin in different groups of treated rats, respectively

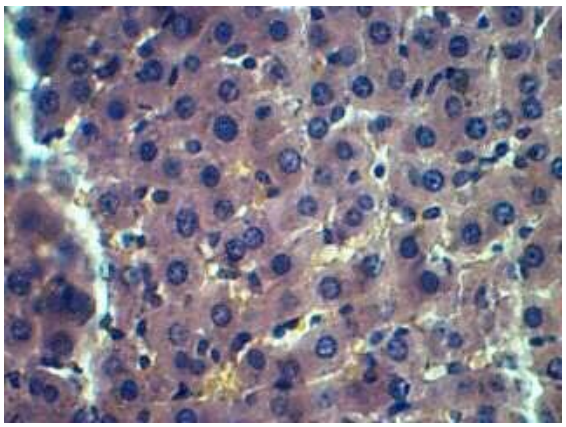

Fig. 2: Section of liver in normal control group rat

Histopathological studies: In control group vehicle treated rats shows normal hepatocytes without any necrosis and vacuolization (Fig. 2). Histopathological examination of the liver section of the rats treated with CCl_4 showed an intense centrilobular necrosis and vacuolization (Fig. 3). The rats treated with Liv 52 and methanolic extract of *Tephrosia calophylla* (150 and 300 mg kg^{-1}) showed a good sign of protection against the CCl_4 to considerable extent as it was evident from the formation of normal hepatic cords and absence of necrosis and vacuoles (Fig. 4-6). Figure 7 and 8 revealed that the liver of these animals not showing any damage during extract treatment.

Liver related disease and disorders is a major problem in world wide and we don't have a good medicine to treat these problems from allopathic medical practice. Available drugs also having toxic effects against liver. Drugs from herbals and any other natural sources might be a reliable source and many plant related drugs play a major role in the management of liver diseases (Subramoniam *et al*., 1998). Previous studies have demonstrated the use of CCl_4 to successfully induce hepatotoxicity in experimental animals (Okuno *et al*., 1986). The proposed mechanism is followed.

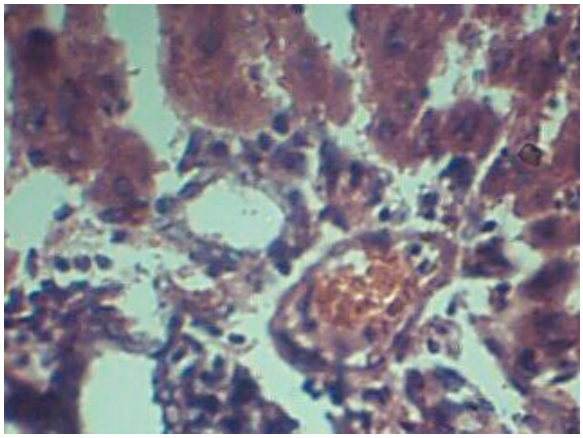

Fig. 3: Section of liver in toxicant group rat (CCl_4)

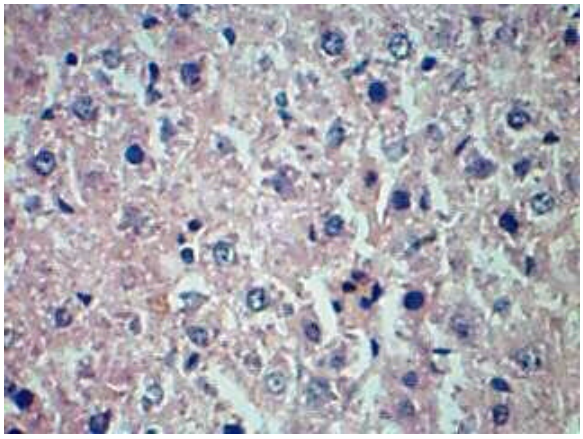

Fig. 4: Section of liver in standard rat (Liv 52 + CCl_4)

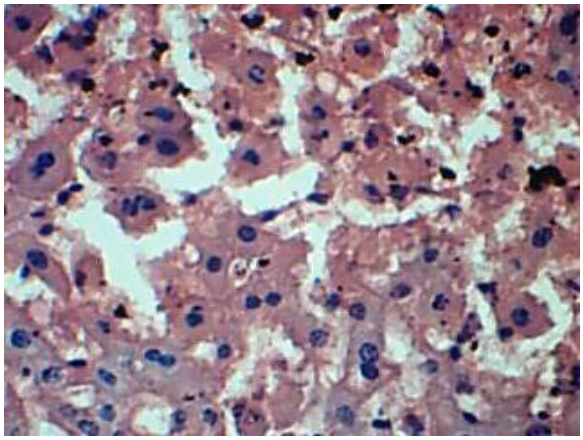

Fig. 5: Section of liver in extract treated rat (*Tephrosia calophylla* 150 mg kg^{-1} + CCl_4)

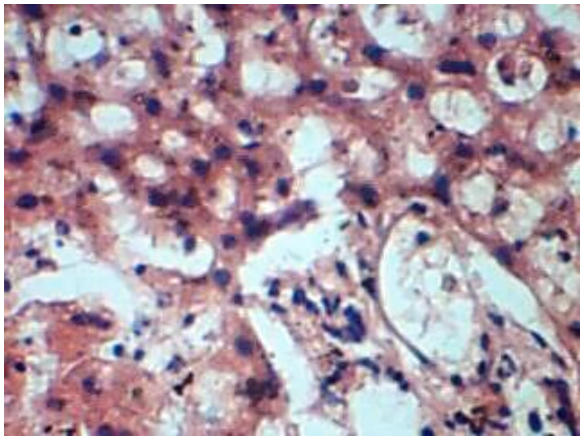

Fig. 6: Section of liver in extract treated rat (*Tephrosia calophylla* 300 mg kg^{-1} + CCl_4)

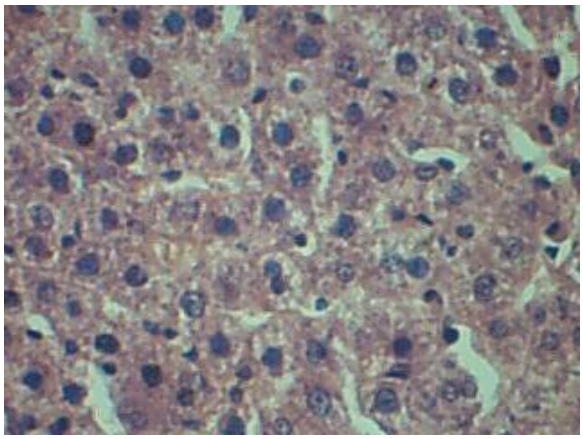

Fig. 7: Section of liver in extract control rat (*Tephrosia calophylla* 150 mg kg^{-1})

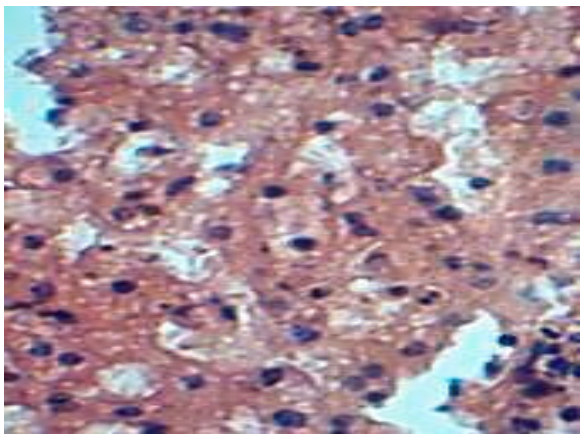

Fig. 8: Section of liver in extract control rat (*Tephrosia calophylla* 300 mg kg^{-1})

CCl_4 induced hepatic damage is due to its Cytochrome P- 450 enzyme system catalyzed hepatic conversion into highly reactive trichloromethyl radical (CCl*), which upon reaction with oxygen radical gives trichloromethyl peroxide radical ($OOCCl^{\circ}{}_3$*). This radical forms covalent bond with sulphydryl group of several membrane molecules like glutathione, which is considered as the initial step in the chain of events leading to lipid peroxidation and hepatic tissue destruction (Brattin *et al*., 1985; Recnagel *et al*., 1989; Ahmed *et al*., 2000; Lee *et al*., 2004; Wang *et al*., 2004). Analysis of liver function can be made by the level of circulating SGOT and SGPT. Normally these levels are high in the liver cytoplasm and whenever the damage occurred which leaked in to serum (Mitra *et al*., 1998). In this model the liver damage is evidenced by an elevation in the serum marker enzymes namely SGPT, SGOT, ALP and total bilirubin. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effects or restoring the normal hepatic physiology, which has been distributed by hepatotoxins. The Liv 52 and the methanolic extract of *Tephrosia calophylla* significantly decreased the CCl_4 induced elevated levels of the enzymes in the treatment group, indicating the enhancement of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. Decrease in the bilirubin after treatment with *Tephrosia calophylla* indicated the effectiveness of the extract in the normal functional status of the liver. Similar findings were made earlier by other researchers in medicinal plants like leaves of *Melia azedarech* and seeds of *Piper longum* (Samudram *et al*., 2008), *Chamomile recutita* (Misra *et al*., 2006) and *Ficus carica* (Venkatesh *et al*., 2007). Histopathological analyses were good in agreement with the biochemical changes. Many research findings. Therefore, there is a possibility that the extract of *Tephrosia calophylla* may possess hepatoprotective activity.

CONCLUSION

In conclusion, the present study demonstrated that the *Tephrosia calophylla* possesses hepatoprotective activity. In addition, the hepatoprotective property may be attributed to the active principles of the plant namely, flavonoids, tannins and other polyphenolic compounds. Further study is warranted to isolate, characterize and screen the active principles from the *Tephrosia calophylla* that possess hepatoprotective activity.

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