

HEPATOPROTECTIVE ACTIVITY OF *JASMINUM ANGUSTIFOLIUM*
LINN. AGAINST CCl₄ INDUCED HEPATIC INJURY IN RAT.

Joshi M C¹, Raju A, Arulanandham A, Saraswathy GR.

¹Manipal College of Medical Sciences, Pokhara, Nepal

²KM COLLEGE OF PHARMACY, Madurai, India

Summary

To evaluate the hepatoprotective effect of ethanolic, chloroform extract of *Jasminum angustifolium* Linn. against CCl₄ induced hepatic damage in rat. The liver damage is assessed by parameters such as alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), cholesterol, glucose, total protein and bilirubin concentration in blood. Hepatic damage is evidenced by rise in the level of alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), cholesterol, glucose, total protein and bilirubin concentration in blood. Liver enzyme values are brought back to nearly normal in animals treated with ethanolic extract of *Jasminum angustifolium* Linn. Values of normal rats for AST, ALT, ALP, glucose, cholesterol, total proteins and bilirubin are 158.31±0.63, 91.83±0.74, 148.41±0.54, 147.45±0.54, 72.56±0.61, 5.46±0.18, and 0.69±0.01 in IU/L respectively. Where in CCl₄ treated rat the values rose to 252.21±0.86, 172.54±0.51, 178.29±0.64, 188.52±0.61, 111.92±5.32, 2.65±0.16 and 1.37±0.014 in IU/L respectively. In, Silymarin treated rats the AST, ALT, ALP, glucose, cholesterol, total protein and bilirubin values are 160.39±0.63, 95.39±0.51, 146.53±0.51, 152.35±0.64, 101.63±0.53, 6.23±0.09 and 0.64±0.01 in IU/L respectively. While in plant ethanolic extract administered rat showed nearly normal values of AST, ALT, ALP, glucose, cholesterol, total protein and bilirubin i.e. 172.36±0.51, 98.61±0.55, 152.24±0.37, 155.60±0.54, 73.46±0.46, 4.76±0.04 and 0.65±0.01 in IU/L respectively. All these values are expressed by means ± SEM. The plant extracts correct all the elevated levels of liver enzymes into normal, of these two extracts ethanolic extract showed better result than chloroform, hence the plant *Jasminum angustifolium* Linn. has potent hepatoprotective effect against CCl₄ induced liver damage in rat.

Keywords: Hepatoprotective; *Jasminum angustifolium* Linn.; Silymarin, CCl₄

Address of Corresponding Author

Dr M C Joshi, MD

Assistant Professor, Pharmacology

Manipal College of Medical Sciences,

Pokhara, Nepal, E-mail- drjoshimukesh@gmail.com

Introduction

Jasminum angustifolium Linn. belonging to the family Oleaceae is distributed in south India (kerala, Karnataka) on the hills of lower elevation. *Jasminum angustifolium* (Linn.) Wild, has very limited systematically carried out investigations. It is called Wild jasmine in English, Banmallika in hindi, Kattumulla, Kattumallika in Malayalam, and Kattumalligai, Kattumullai in Tamil. The traditional systems of Siddha and Ayurvedic medicine use this plant alone or in combination with other medicinal plant for the treatment of various diseases [2]. The roots are bitter, acrid and are useful for external application in ringworm and herpes infestations and are recommended for ophthalmopathy, ulcerative stomatitis, leprosy, pruritus and wounds. The leaves are used as an emetic in cases of poisoning [3]. Hence this study was planned to evaluate the effect of ethanolic and chloroform extract of leaves and roots of *Jasminum angustifolium* Linn. against CCl₄ induced liver damage in rat.

Materials And Methods

Collection of plants

The whole plant of *Jasminum angustifolium* was collected from kanyakumari district which is the southern most districts in Tamil Nadu. The district lies between 77°15' and 77°36' of the eastern longitudes and 8°03' and 8°35' of the northern latitudes. This plant was authenticated by Dr. Stephan, Department of Botany, The American College, Madurai.

Extraction

The shade dried whole plant of *Jasminum angustifolium* Linn. were powdered coarsely and about 750g of this powder was extracted (soxhlet) with 70% ethyl alcohol and chloroform, the yield was 22.4g of ethanolic extract of *Jasminum angustifolium* (EEJA) and 4.8g of chloroform extract of *Jasminum angustifolium* (CEJA) respectively. The extract was dried in vacuum and re-suspended in water before use. The phytochemical screening proves the presence of carbohydrate, glycosides and flavonoids [4], [5].

Animals

Albino wistar rats of either sex, weighing 200-250g, were housed under standard environmental conditions of temperature, humidity and were provided with standard rat chow and water ad libitum.

Induction of hepatic injury

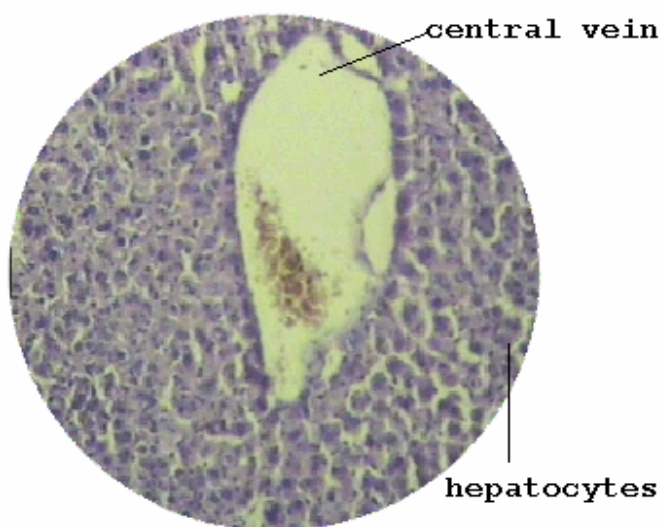
The rats were subjected to oral administration of 1ml/kg CCl₄ mixed with an equal volume of liquid paraffin, twice a week for 28 days. Three days after the last dose, the rats were sacrificed under anesthesia. Blood and liver samples were collected for biochemical and histopathological studies.

Treatment protocol

The animals were divided into five groups of six animals each and treated orally as per the following schedule

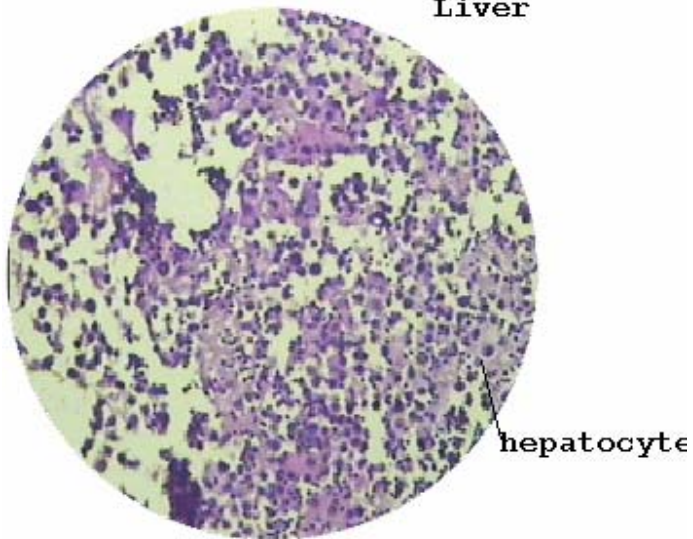
Normal control group (G1): The animals were treated with distilled water for 28 days.

PLATE NO 1: NORMAL RAT LIVER



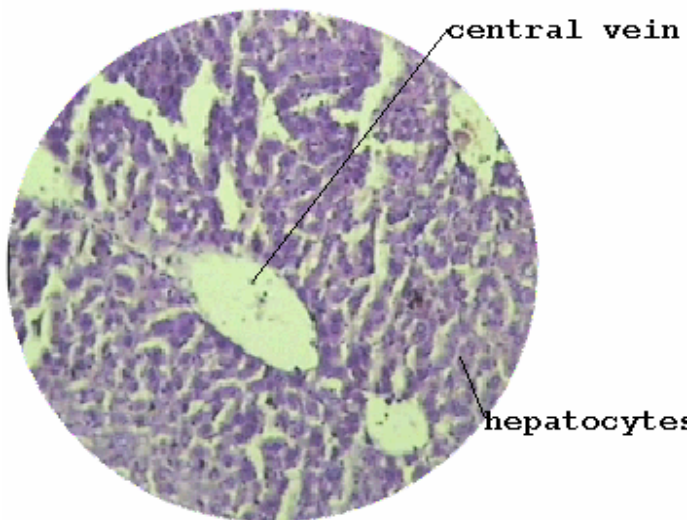
CCl₄ control group (G2): The animals were treated with CCl₄ twice a week for 28 days (1ml/kg CCl₄ mixed with equal quantity of liquid paraffin) the remaining days they received distilled water.

PLATE NO 2: CCl₄ CONTROL RAT LIVER
Liver



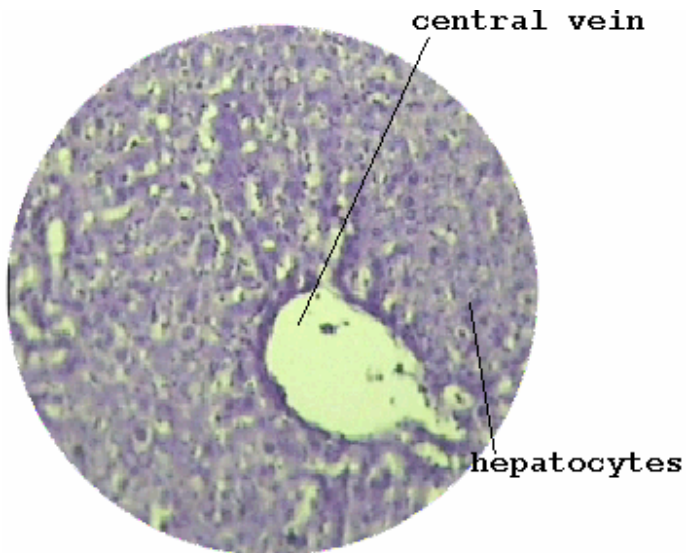
Silymarin control group (G3): The animals were treated with CCl₄ twice a week for 28 days (1ml/kg CCl₄ mixed with equal quantity of liquid paraffin), with Silymarin 0.007g/kg for 28 days.

PLATE NO 3: STANDARD (SILYMARIN) LIVER



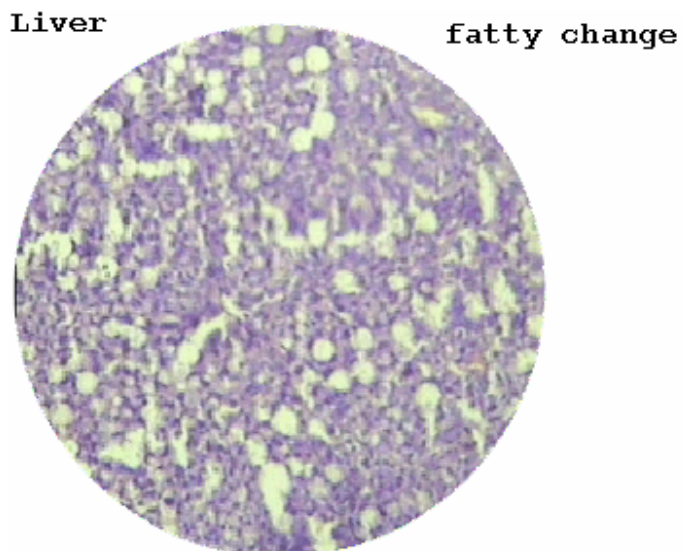
EEJA group (G4) : The animals were treated with CCl₄ twice a week for 28 days(1ml/kg CCl₄ mixed with equal quantity of liquid paraffin), with 350mg/kg of ethanolic extract of plant daily for 28 days.

PLATE NO 4: EEJA TREATED LIVER



CEJA group (G5): The animals were treated with CCl₄ twice a week for 28 days (1ml/kg CCl₄ mixed with equal quantity of liquid paraffin), with 350mg/kg of chloroform extract of plant daily for 28 days.

PLATE NO 5: CEJA TREATED LIVER



After 28 days the animals were left free for 3 days and then were sacrificed.

The animals were made to fast overnight after the experimental period. They were anaesthetized with ether and the blood was collected by carotid artery bleeding. From that serum cholesterol, AST, ALT, ALP, glucose, total protein and bilirubin were evaluated.

Livers were dissected out, weighed and preserved for histopathological studies. The livers from different groups were weighed and differences in weights were noted.

Histopathological studies:

Liver sections were preserved in 10% formalin. They were stained with haematoxylin and eosin, the stained sections were observed under the microscope to estimate the extent of liver damage.

Statistical analysis

All values are expressed as means \pm SEM. The results were calculated and subjected to Analysis of variance (ANOVA) followed by students Newman Keul's Multiple range test. P values >0.05 were considered significant.

Results

Food consumption and weight gain

We observed that there was significant decrease in body weight of CCl₄ treated group as compared to normal control group. Treatment of rats with Silymarin and plant extracts showed significant increase in body weight as compared to CCl₄ treated group. The livers from different groups were weighed and differences in weights were noted (Table 1)

(Table 1) Effect of EEJA and CEJA on body weight and liver weight.

S.No	BODY WEIGHT	LIVER WEIGHT
G ₁	8.51 \pm 1.718	6.59 \pm 0.57
G ₂	4.54 \pm 0.675*** ^a	7.36 \pm 0.23*** ^a
G ₃	8.21 \pm 1.03	6.35 \pm 0.89
G ₄	8.18 \pm 1.505*** ^b	6.21 \pm 0.24*** ^b
G ₅	7.66 \pm 1.151** ^b	6.06 \pm 0.57** ^b

G₁ - Normal Control, G₂ – CCl₄ Control, G₃ – Standard (Silymarin), G₄- EEJA, G₅-CEJA. All value is expressed as mean \pm SEM for 6 animals in each group. a - Values are significantly different from control (G₁); b – Values are significantly different from cancer control (G₂); * P (<0.05), ** P (<0.01), *** P (<0.001); All values are found out by using one way ANOVA followed by Newman Keul's Multiple range test.

Serum marker enzymes

All the marker enzymes, viz., AST, ALT, ALP, glucose, cholesterol, total protein and bilirubin levels showed abnormal value in CCl₄ induced liver damage when compared to control group and are reversed by the standard and the plant extracts significantly.(Table 2)

Table 2 : Effect of EEJA and CEJA on Liver enzymes and Lipid profile

Groups	AST	ALT	ALP	Glucose	Cholesterol	Total protein	Bilirubin
G ₁	158.31 ±0.63	91.83 ±0.74	148.41 ±0.47	147.45 ±0.54	72.56 ±0.61	5.46 ±0.18	0.69 ±0.01
G ₂	252.21 ±0.86*** ^a	172.54 ±0.51*** ^a	178.29 ±0.64*** ^a	188.52 ±0.61*** ^a	111.92 ±5.32*** ^a	2.65 ±0.16*** ^a	1.37 ±0.01*** ^a
G ₃	160.39 ±0.63	95.39 ±0.51	146.53 ±0.51	152.35 ±0.64	101.63 ±0.53	6.23 ±0.09	0.64 ±0.01
G ₄	172.36 ±0.51*** ^b	98.61 ±0.55*** ^b	152.24 ±0.37*** ^b	155.60 ±0.54*** ^b	73.46 ±0.46*** ^b	4.76 ±0.04*** ^b	0.65 ±0.01*** ^b
G ₅	182.42 ±0.58*** ^b	99.27 ±0.86*** ^b	157.72 ±0.53*** ^b	166.36 ±0.63*** ^b	82.62 ±0.59*** ^b	4.1 ±0.05*** ^b	1.01 ±0.02*** ^b

G₁ - Normal Control, G₂ – CCl₄ Control, G₃ – Standard (Silymarin), G₄- EEJA G₅- CEJA

All value is expressed as mean ±SEM for 6 animals in each group.

a - Values are significantly different from control (G₁)

b – Values are significantly different from cancer control (G₂)

* P (<0.05), ** P (<0.01), *** P (<0.001)

All values are found out by using one way ANOVA followed by students

Newman Keul's Multiple range test.

Discussion

Carbon tetrachloride is one of the most commonly used hepatotoxin which causes liver fibrosis. It is well documented that carbontetrachloride is biotransformed under the action of cytochrome P-450 in the microsomal compartment of liver to trichlomethyl radical which readily reacts with molecular oxygen to form trichloromethyloeroxy radical[6]. This free radical in the presence of oxygen may cause peroxidation of lipid on target cell resulting in extensive damage [7]. Hepato toxic effect of CCl₄ is due to oxidative damage by free radical generation [8] and anti oxidant property is claimed to be one of the mechanism of hepatoprotective drugs. Many phytochemical reports revealed that the ethanolic extract of the plants were found to contain higher concentration of flavonoids and glycosides.[9] and it has been reported that the flavonoid constituents of plant possess antioxidant properties by free radical scavenging [10]. Administration of CCl₄ (1ml/kg CCl₄ mixed with equal quantity of liquid paraffin) to rats produced hepatotoxicity showed by significant increase in the serum levels of AST, ALT ,ALP, Glucose , Cholesterol and Bilirubin in comparison to control group. Also total protein levels were significantly decreased to 2.65g/dl in CCl₄ control groups from the level of 5.46g/dl in normal control group as shown in the table 2. Ethanolic and chloroform extract of *Jasminum angustifolium* Linn. given at dose 350mg/kg , reduce the raised serum enzyme levels get back to nearly normal but also improved serum lipid profile. The results are well comparable with Silymarin (standard drug). In conclusion, the results of present study show that *Jasminum angustifolium* Linn. has potent hepatoprotective activity against carbontetrachloride induced liver fibrosis in rats.

REFERENCES

1. Burt AD, Cellular and Molecular Aspect of Hepatic Fibrosis, J Pathol, 1993, 171,105-114.
2. Deni Bown, The Royal Horticultural Society: Encyclopedia of Herbs and Their Uses.1995, 144.
3. Warriar, P.K, Nambiar, V.P.K, Ramankutty, C, Indian Medicinal Plants, Vol 3 , Orient longman, Madras , 1995, 242.
4. Harborne, J.B, Phytochemical Methods. Chapman and Hall Ltd., London, 1973, 52-105.
5. Wagner, H, Bladt, S, Zagaiwski, E.M, Plant Drug Analysis. Springer- Verlag, Berlin/New York, 1984, 126-169.

6. Raucy JL, Lasher J. Bioactivation of halogenated hydrocarbons by cytochrome P-450 E, Crit Rev. Toxicol 1993, 23:1-20.
7. Recknagel R.O. Trends Pharmacol Sci 1983; 4:129.
8. Recknagel R.O, Carbon tetrachloride hepatotoxicity, Pharmacol rev, 1967, 19, 145-208.
9. Harboni JB Phytochemical methods, In: A guide to modern techniques of plant analysis, Chapman& Hall, London, 1973, pp.1.
10. Hesham R, EI-Seedi and Shgeru Nishiyama, Indian J. Pharm Edy, 2002, 36,191-194.