Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 12, pp.63449-63452, December, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

IN VIVO HEPATOPROTECTIVE ACTIVITY OF ROOT AQUEOUS EXTRACT OF VEN SIVATHAI VER CHOORNAM (*OPERCULINA TURPETHUM*) IN CCl_4 INDUCED HEPATOTOXICITY IN RATS

[1]Dr. Lakshmanaraj, C., [2]Dr. Kanagavalli, K., [3]Dr. Rajammadevi Sorubarani, K., [4]Dr. Karolin Daicy Rani, R. and [5,*]Dr. Sankaranarayanan, S.

[1,3,4] Department of Gunapadam,Govt Siddha Medical College, Chennai, Tamilnadu
[2]Department of Medicine, Govt. Siddha Medical College, Chennai, Tamilnadu
[5]Assit. Professor, Department of Medicinal Botany, Goverment Siddha Medical College, Arumbakkam, Chennai-600 078

ARTICLE INFO

Article History:
Received 13th September, 2017
Received in revised form
03rd October, 2017
Accepted 16th November, 2017
Published online 31st December, 2017

Key words:
O. turpethum,
Ven sivathaiverchoornam,
Hepatoprotective activity.

ABSTRACT

A simple and effective RP-HPLC method The study was considered to assess the hepatoprotective activity of pre-treatment with aqueous extract of Ven sivathaiverchoornam (*O. turpethum*) against carbon tetrachloride-induced hepatotoxicity in Wistar rat model. Liver damage was induced in experimental animals by administering CCl_4. The aqueous extract of *O. turpethum* (60 and 100 mg/kg, po) was given for five days. Silymarin (100 mg/kg, po) was specified as the reference drug. Hepatoprotective result was recorded by assaying the activities of serum marker enzymes like SGPT, SGOT, ALP, and bilirubin and cholesterol. The actions of all the marker enzymes recorded a significant elevation in CCl_4 treated rats, which were significantly recovered towards an almost normal level in animals administered with aqueous extract of *O. turpethum*root at a dose of 60 and 100 mg/kg. Aqueous extract of *O. turpethum* root not permitted decrease in the excretion of ascorbic acid in CCl_4 induced hepatotoxicity in rats. The results indicate that aqueous extract of *O. turpethum* possess hepatoprotective property. This property may be attributed to the related flavonoids, terpenoid and phenolic compounds present in the root of *O. turpethum*.

Copyright © 2017, Lakshmanaraj et al. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Citation: Dr. Lakshmanaraj, C., Dr. Kanagavalli, K., Dr. Rajammadevi Sorubarani, K., Dr. Karolin Daicy Rani, R. and Dr. Sankaranarayanan, S. 2017. "In vivo hepatoprotective activity of root aqueous extract of ven sivathai ver choornam (*operculina turpethum*) in ccl_4 induced hepatotoxicity in rats", *International Journal of Current Research*, 9, (12), 63449-63452.

INTRODUCTION

In Siddha system of medicine use of plants in the treatment of various ailments for thousands of years. The herbal plants initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. Many of the currently available drugs were derived either directly or indirectly from medicinal plants. Recent interest in natural therapies and alternative medicines has made researchers pay attention to traditional herbal medicine. In the past decade, attention has been centered on scientific evaluation of traditional drugs with plant origin for the treatment of various diseases. Due to their effectiveness, with presumably minimal side effects in terms of treatment as well as relatively low costs, herbal drugs are widely prescribed, even when their biologically active constituents are not fully identified (Levy et al., 2004).

Corresponding author: **Dr. Sankaranarayanan, S.,**
Assit. Professor, Department of Medicinal Botany, Goverment Siddha Medical College, Arumbakkam, Chennai-600 078.

Liver diseases which are still a global health problem maybe classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in liver fibrosis). Unfortunately, treatments of choice for liver diseases are controversial because conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects. The utility of natural therapies for liver diseases has a long history. Despite the fact that most recommendations are not based on documented evidence, some of these combinations do have active constituents with confirmed antioxidant, anti-inflammatory, ant carcinogenic, ant fibrotic, or antiviral properties. Although a large number of these plants and formulations have been investigated, the studies were mostly unsatisfactory. For instance, the therapeutic values, in most of these studies, were assessed against a few chemicals-induced subclinical levels of liver damages in rodents. The reasons that make us arrive at such a conclusion are lack of standardization of the herbal drugs, limited number of randomized placebo controlled clinical trials, and paucity of traditional toxicologic evaluations

(Thyagarajan et al., 2002). *Operculinaturpethum* syn. *Ipomoea turpethum* is a stout perennial climber that exudes a milky juice when cut, with long fleshy roots and long twisting pubescent stems that are angled, winged which become very tough and brown when old. The leaves are simple, pubescent on both sides and variable in shape, cordate or truncate at base 5-10 cm long and1.3- 7 cm wide. The flowers are white, campanulate, sepals long, borne in cymes of few flowers, giving way to globose capsules enclosed within overlapping brittle sepals. The capsules is rounded, being 1 to 1.5 centimeters in diameter, and contains normally 4 black, smoothseeds. In Siddha, root of *O.turpethum*is used internally totreat fevers, anorexia, edema, anemia, constipation, hepato-splenomegaly, hepatitis, intoxication, abdominal tumors, ulcers, wounds, worm infestation, pruritus and other skin disorders. Root powder of *O.turpethum* mixed with ghee and honey is also used to treathematemesis, tuberculosis & herpes (Nadkarni and Nadkarni, 2007).

MATERIALS AND METHODS

Plant material

Roots of the plant *Operculinaturpethum* were collected (in the month of August) from the surrounding fields of Chengalpet, Kanchipuram district, Tamilnadu.The identification of plant was made by Dr.S. Sankaranarayanan, Head, Department of Medicinal Botany, Government siddha medical college, Arumbakkam, Chennai, Tamilnadu. The voucher specimen (Ref No:33/MB) of the plant material has beendeposited in the Department of Medicinal Botany.

Preparation of extract

The roots of the plant *O.turpethum* were washed thoroughly in tap water, shade dried and powdered. The coarse powder was soaked in boiling distilled water and left for 2 hours at room temperature, the mixtures were filtered and cooled overnight at - 4 °C and concentrated extracts were freeze dried (Komolafe *et al,* 1988) The yields percentages were calculated and the residue obtained was kept in a refrigerator for future use.

Phytochemical analysis of *Operculinaturpethum*

The aqueous extract of *O. turpethum* was freshly prepared and various chemical constituents were analysed according to methods described by Allen (1974) and Harbone (1976). The different chemical constituents tested for included tannins, saponin, glycosides, alkaloids, terpenoids, anthocynin, polyphenol and flavonoids.

Animals

Wister Albino rats (150 - 200 g) and Albino mice (20 – 25g) of either sex procured from Bioneeds animal house, Dhavas pet, Tumkur, were used for the study. Theanimals were kept in polypropylene cages and maintained at a temperature of 25 ± 2ºC. They were fed with standard diet supplied by Hindustan Lever Pvt Ltd., Bangalore. The study has obtained the approval (Ref: IAEC/PP/08/2006-2007) from the Institutional Animal Ethical Committee (IAEC). All the animal experiments are conducted in accordance with the guidelines of the CPCSEA (Reg No.), guide for care and use of laboratory animals. After procuring the animals were acclimatized for 10 days under standard husbandry conditions as: Relative humidity 45 - 55%, and 12h light and dark cycle.

Acute toxicity study

The albino mice of 20 – 25 g body weight of either sex were selected to find out the acute toxicity study of aqueous extract of *O. turpethum* root. The dose of 5,50, 300 and 2000 mg/kg were selected based on the fixed dose (OCED Guideline No. 420) method of CPCSEA. The extract was administered by intraperitonially. The animals were continuously observed for 24 h to detect changes in autonomic or behavioral responses. Mortality in each group was observed for 7 days.

Assessment of hepato protective activity

The animals were separated into six groups of six Wistaralbino rats each. The animals were fasted for 24 h priorto carbon tetrachloride treatment. Group I was maintained as normal control received normal saline 5 ml/kgorally. All the animals of group II to VI received carbon tetrachloride diluted with olive oil (1:1) at dose of 1 ml/kg, subcutaneously for two successive days (2nd and 3rd day).Group II animals were maintained as carbon tetrachloride control without any drug treatment. Group III, IV and Vanimals were treated with 40, 60 and 100 mg/kg aqueous extract of *O. turpethum* rootrespectively by or all route. Group VI animals were treated with Silymarin (100 mg/kg, orally) which served as standard group. The vehicle or drug treatment was carried out orally from 1stday to 5thday with concurrent administration of carbon tetrachloride on 2nd and3rd day. During the period of drug treatment the rats were maintained under normal diet and water *ad libitum*. The animals of all the groups were sacrificed by light ether anesthesia on 6th day. The blood sample of each animal was collected separately by carotid artery into sterilized dry centrifuge tubes and allowed to coagulate for 30 min. Serum was separated by centrifugation 3000rpm for 15 min. The serum was used to estimate serumglutamate pyruvate transaminase (SGPT), serum glutamate oxaloactetate transaminase (SGOT), serum direct bilirubin and total bilirubin, serum alkaline phosphatase (ALP), serum triglycerides and serum cholesterol (Henry etal., 1974; Gambino, 1965; Walter and Schutt, 1974;Fossatiand Principle, 1982; Deeg andZiegenhorn, 1983). Livers were removed and preservedin 10% formalin solution for histopathological studies.

Statistical analysis

The mean ± S.E.M. was calculated for each parameter. Total variations, present in a set of data were estimated by one way analysis of variance (ANOVA), followed by Dunne test. $P<0.05$ was considered as statistically significant when compared to control group. The percentage of the protection is calculated as 100 X (Values ofCCl_4 control – Values of test sample) / (Values of CCl_4control – Values of normal control).

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of aqueous extract from the root of *O. turpethum* was considered. The aqueous extract contains saponins, flavonoids, tannins, alkaloid, terpenoid, glycosidesand absence of polyphenol, anthocynin. Rajashekar et al., (2006) previously reported that root bark of *O. turpethum* was rich sources of turpeth resin, which consisting of 10% ‘turpethin’ and also glycoside analogue of Jalapine and Convolvulin.

Table 1. Phytochemical screening of aqueous extract from the root of *O. turpethum*

S.No.	Phytochemical Constituents	Result indicated	Aqueous extract from the root of *O. turpethum*
1.	Alkaloids Dragendroffs reagent	Brown precipitation	+
	Mayearsreagent	Yellow precipitation	+
2.	Flavonoids Alkalaine test	Yellow coloration	+
	Lead acetate	Immediate Precipitation	+
3.	Polyphenols Ferrozine Test	Blue Coloration	-
4.	Terpenoids Salkowski test	Brown ring	+
5.	Tannins	Dark green blue	+
6.	Glycosides Keller-Killani test	Reddish brown ring	+
	Bronbagers Test	Pink colour in ammonia layer	+
7.	Saponins Froth Test	Foam	+
8.	Anthocynin Ammonia Test	Yellow colour in ammonia layer	-

-- Negative (absent); + Positive (present)

Table-2. Effect of aqueous extract of *O. turpethum* on repeated oral toxicity for 15 days

Groups	Hb (gm/100ml)	RBC (millions/cu.mm)	WBC (cells/cu.mm)	Differential leucocyte count (%)		
				Lymphocytes	Monocytes	Granulocytes
Control	12.55±0.65	5.12±0.78	5468.33±262.78	77.00±3.89	5.50±1.04	17.50±4.27
Aqueous extract of *O .turpethum*	12.45±0.59^{ns}	4.57±0.52^{ns}	5476.66±306.37^{ns}	77.16±2.92^{ns}	5.33±1.75^{ns}	17.5±4.27^{ns}

N=6; Values are expressed as mean ± S.D followed by Students Paired 'T' Test
Ns – non significant when compared to control

Table-3. Effect of aqueous extract of *O. turpethum* on Marker enzyme levels of Liver and Kidneyafter 15 days repeated oral dose

Groups	ALP (K.A.Units)	AST (IU/L)	ALT (IU/L)	Urea (mg/100ml)	Bilirubin (mg/dl)
Control n=6	2.79±0.39	74.44±3.10	26.42±1.65	11.60±0.93	5.39±0.41
Aqueous extract of *O.turpethum* (100mg/kg,po)	2.90±0.46 ^{ns}	75.23±4.81 ^{ns}	26.44±2.10 ^{ns}	11.70±0.79 ^{ns}	5.40±0.40 ^{ns}

N=6; Values are expressed as mean ± S.D followed by Students Paired 'T' Test
Ns – non significant when compared to control

Table 4. Effects of aqueous extract of *O. turpethum* on certain serum biochemical parameters in CCl_4 induced hepatotoxicity in rats

Drug treatment	ALP (KA units)	AST (IU/L)	ALT (IU/L)	SGPT (IU/L)	SGOT (IU/L)	Cholesterol (mg/dl)	Bilirubin (mg/dl)
CCl_4 treated rats	66.35±6.59	61.80±2.55	52.30±2.3	214.07±2.69**	218.18±2.45**	278.44±2.96	0.3357±0.0022**
Aqueous extract of *O .turpethum* (60mg/kg,po)	60.28±5.36	58.24±1.48	35.15±2.8	98.27± 1.88**	100.55±1.68**	225.29±1.16**	0.1372±0.0017** (68.73%)
Aqueous extract of *O .turpethum* (100mg/kg,po)	55.90±4.39**	55.30±4.59**	26.91±1.19**	46.58± 0.77**	52.16± 1.02**	201.56±0.50**	0.0275±0.0015** (98.28%)
Silymarin	48.70 ±.61**	53.20 ±.03**	27.17±1.09 **	46.10± 1.08**	50.23± 1.03**	195.16±1.45**	0.0298±0.0022** (94.59%)

Values are Mean ± SEM, (n = 6 in each group). Figures in parenthesis are percent protection as compared to CCl_4 control. CCl_4 control group was compared with normal group and all values were significantly different (P< 0.01). Experimental groups were compared with CCl4 control: *P<0.05 and **P<0.01, ns = non significant.

In an acute toxicity study of *O. turpethum*were not found to be toxic at a dose of 2000 mg/kg, intra peritoneally. *O. turpethum* administration (90mg/kg) for15days in rats did not exhibit evidence of toxicity (Table-2). An aqueous extract of *O.turpethum*, when administered in different groups of Wistar rats of either sex in doses ranging from 90mg/kg, not produced lethality in any of the groups. Also the extract did not produce any alterations in liver function markers like SGOT, SGPT, serum alkaline phosphatage and serum bilirubin (Table-3).Suresh Kumar et al.,(2006) have been reported that acute toxicity study of ethanol extract of *O.turpethum*, when administered in different groups of Wistar rats of either sex in doses ranging from 100-2000 mg/kg, produced nolethality in any of the groups. Also the extract did not produce any alterations in liver function markers like SGOT, SGPT, serum alkalinephosphatage and serum bilirubin.

Effect of aqueous extract of *O. turpethum* on CCl_4induced liver damage in rats with reference to biochemical changes in serum are shown in Table-4. The 5th day treatment, blood sample collected from of CCl_4 treated and control animals. The result of biochemical showed significant increase in the level of SGPT, SGOT, ALP, triglycerides and cholesterol when compare to normal control. Pretreatment with aqueous extract of *O. turpethum* at 60 and 100 mg/kg showed marked decreased of SGPT, SGOT, ALP, triglycerides and cholesterol as compared to the CCl_4 treated group. The maximum protection was shown by aqueous extract of *O. turpethum* at the dose of 100 mg/kg body weight (Table-4). Bilirubin level was shown in Table 4.The rats treated to CCl_4 showed significant increased amount of bilirubin as compare to control. Pretreatment with aqueous extract of *O. turpethum* showed significant (P < 0.01) decreased level of total and

direct bilirubin to the near normal which is comparable to the values registered inthe standard drug treated (Silymarin) group of animals, indicating the protection of hepatic cells. Protection against CCl_4induced hepatic damage at 60 mg/kg dose of extract was negligible in all these biochemical markers. The present study also revealed that the specified dose of CCl_4 (1ml/kg, sc) produced significant elevation in SGPT, SGOT, ALP, bilirubin, and cholesterol indicating all impaired liver function and these parameters have been reported to sensitive indicator of liver injury (Salvi et al., 2001).The aqueous extract of *O. turpethum* when administered orally to rats showed a significant dose dependent hepatoprotective activity at 60 and 100 mg/kg. The extracts at 60 mg/kg does mild alter the enzymesmarker intoxicated group. A very important study with this *O. turpethum* extract at dose of 100 mg/kg isgreatly efficient in lessening the prominent level of serumtotal bilirubin.

Conclusion

It is concluded that treatment with aqueous extract of *O. turpethum* decreases the CCl_4-induced elevation in biochemical parameters. These results recommend that the aqueous extract of *O. turpethum* was effective in bringing about functional enhancement of hepatocytes. The curative effect of this extract was also definite by histological observations. The study revealthat, root of *O. turpethum* contain tannin, terpenoids, flavanoids and phenolic compounds may have a potential therapeutic approach to hepatoprotective properties.

REFERENCES

Allen ST. 1974. Chemical analysis of ecological material. Blackwell Scientific Publication, New York 313.

Deeg R., Ziegenhorn J, 1983. Kinetic enzymatic method for automated determination of total cholesterol in serum, Clin Chem. 29: 1798- 1802.

Fossati, P., Principle, L. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide, ClinChem 28: 2077-2080.

Gambino, S.R. 1965. *Standard method of Clinical Chemistry*, (Academic Press, New York) p. 55.

Harbone JR. 1976. Phytochemical methods. A guide to modern techniques of plant analysis. Charpan and Hall, London; 78.

Henry RJ, Cannon DC, Winkelman JW. 1974. *Clinical Chemistry Principles and Techniques*, (Harper and Row, New york) pp 822.

Komolafe, O. O., Anyabuike, C. P. and Obaseki, A. O. 1988. The possible role of mixed function oxidases in the hepatobiliary toxicity of Azadirachtaindica. Fitoterapia, LIX: (2), 109-113.

Levy C, Seeff LD, Lindor KD. 2004. Use of herbal supplements for chronic liver disease. *ClinGastroenterolHepatol*, **2**: 947-956.

Nadkarni K. M. and Nadkarni A. K. 2007 Ed. Indian MateriaMedica, Vol I, Bombay Popular Mumbai, pp. 691-694.

Rajashekar M, Bhande L, Pramod Kumar, Nitin KM, and RamachandraSetty S. 2006. Pharmacological Screening of Root of *Operculinaturpethum*and its Formulations. ActaPharmaceuticaSciencia, 48: 11-17.

Salvi A, Carrapt P, Tillement J, Testa B. 2001. Structural damage to protein and influence of protein binding, BiochemPharmacol. 61: 1237-1245.

Suresh Kumar SV, Sujatha C, Shymala J, Nagasudha B and Mishra SH. 2006. Protective effect of Root Extract of *Operculinaterpethum*Linn. Against Paracetamol induced Hepatotoxicity in Rats. Indian Journal of Pharmaceutical Sciences, 68 (1): 32-5.

Thyagarajan SP, Jayaram S, Gopalakrishnan V, Hari R, Jeyakumar P, Sripathi MS. 2002. Herbal medicines for liver diseases in India. *J GastroenterolHepatol,* 17: S370-S376.

Walter, K., Schutt, C, 1974. Acid and alkaline phosphatases in serum. In: VerlagChemieWeinheim, In: Hans Ulrich Bergmeyer (Ed.), *Methods of Enzymatic Analysis*, vol. 2. Academic Press Inc., New York, pp. 856– 864.

****