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

International Journal of Current Research
Vol. 8, Issue, 12, pp.42890-42893, December, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ACUTE TOXICITY AND ANTIDIARRHOEAL ACTIVITY OF THE CRUDE ETHANOLIC EXTRACT OF THE *OPERCULINA TURPETHUM*

***,[1]Mohammad Abu Bin Nyeem, [1]Md. Moniruzzaman Khan, [2]Rabiul Islam, B. M., [3]Md. Abu Hossain, [2]Meher Nowrose and [4]Md. Abdul Awal**

[1]Department of Unani Medicine, Hamdard University, Bangladesh
[2]Department of Public Health, ASA University, Bangladesh
[3]Department of Public Health, North South University, Bangladesh
[4]Department of Pharmacology, Bangladesh Agricultural University, Bangladesh

ARTICLE INFO

Article History:
Received 03rd September, 2016
Received in revised form
25th October, 2016
Accepted 29th November, 2016
Published online 30th December, 2016

Key words:

Operculina turpethum,
Antidiarrhoeal activity,
Acute toxicity,
Convolvulaceae.

ABSTRACT

Operculina turpethum is a perennial with milky juice belongs to family Convolvulaceae. This plant was widespread in old tropics from E. Africa to N. Australia and common in Godavari, Andhra Pradesh India & Bangladesh. The objective of the present study was to evaluate the acute toxicity and antidiarrhoeal activity of the ethanolic extract of the *Operculina turpethum* in mice. Ethanolic extract of the *Operculina turpethum* (Linn.) was administered orally in mice at various dose levels to determine the acute toxic effects and the median lethal dose (LD_{50}). Antidiarrhoeal activity was tested by castor oil induced diarrhoea in mice, at the doses of 500 mg/kg body weight comparable to the standard drug loperamide at the doses of 50 mg/kg body weight. The acute toxicity of the ethanolic extract was found to be 5.0 gm/kg body weight within 95 % confidence limits. The mice showed signs of cerebral irritation before dying. Histopathological examinations of the viscera showed necrosis of the liver and kidneys. The antidiarrhoeal effects increased mean latent period and decreased the frequency of defecation significantly ($P<0.05$; $P<0.01$). This study suggested that the extract possess antidiarrhoeal activity. It was concluded that the extract of *Operculina turpethum* is safe and possess antidiarrhoeal activity, however the cerebral mechanism that lead to the death of the mice need to be investigated and find out the active metabolites responsible for antidiarrhoeal activity.

Citation: Mohammad Abu Bin Nyeem, Md. Moniruzzaman Khan, Rabiul Islam, B. M., Md. Abu Hossain, Meher Nowrose and Md. Abdul Awal, 2016. "Acute toxicity and antidiarrhoeal activity of the crude ethanolic extract of the *Operculina turpethum*", *International Journal of Current Research,* 8, (12), 42890-42893.

INTRODUCTION

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhoea. Diarrhoea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the pediatrics ward die of diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and children below 5 years and old especially in developing countries (Fauci *et al*., 1993). The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organizations to control it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used antidiarrhoeal drugs, which are not always free from adverse effects (Hardman & Limberd, 1992). A range of medicinal plants with antidiarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these antidiarrhoeal traditional medicines has not been scientifically evaluated. Plants have almost always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. *Operculina turpethum,* belongs to convolvulaceae is well known in Ayurveda as *Tribit* and *Turbud* in Unani system of medicine, is a perennial stout climber with wringed stem and milky juice and its root is incorporated in the *Avipattikara Churna* (an Ayurvedic preparation used for the treatment of hyperacidity, gastric ulcer and related gastrointestinal disturbances) (Ram Rastogi, 2006). Root of *Operculina turpethum* is used internally to treat fevers, anorexia, edema, anaemia, ascites, constipation, hepatosplenomegaly, hepatitis, abdominal tumors, ulcers, wounds, worm infestation, pruritis, and other skin disorders (Anonymous, 2001). Root is also administered to treat obesity, hemorrhoids, cough, asthma dyspepsia, flatulence, paralysis, gout, rheumatism, melancholia, scorpion sting and snake bites (Sharma and Vidnyana, 2006). The paste of root powder of

****Corresponding author: Mohammad Abu Bin Nyeem,***
Department of Unani Medicine, Hamdard University, Bangladesh

Operculina turpethum is used topically to treat vitiligo and other skin disorders, alopecia, cervical lymphadenitis, hemorrhoids, fistulas, and ulcers (Srikantha Murty, 2008). Oil extracted from the root bark of Trivrit is used in skin diseases of a scaly nature (Alam *et al*., 2010). A processed ghee with *Operculina turpethum* or fresh juice of *Operculina turpethum* leaves is dropped into the eyes to treat diseases like corneal opacity or ulcer and conjunctivitis. Root powder of Trivrit mixed with ghee and honey is also used to treat haematemesis, tuberculosis, and herpes. Root bark, root stem and leaves of this herb have high medicinal value (Bapalal Vaidya, 2005). It is one of the plants mentioned in the literature having claims of activity against liver disorders (Jain and Saxena, 1987). It also has antioxidant, anticancer (Austin, 1982) hepatoprotective (Kumar *et al*., 2006) antidysmenorrheal (Jahan and Sujatha, 2010) anthelmintic, expectorant, antipyretic, analgesic, anti-inflammatory and purgative properties. It contains a wide variety of phyto constituents, which are useful in treatment of different ailments and includes glycosidic resin, coumarins, beta-sitosterol, and essential oils (Deeaph and Malti, 1994). The root bark of *Operculina turpethum* is rich in turpethum resin consisting of 10% 'turpethin' which is a glycoside analogue of Jalapine and Convolvulin and is insoluble in ether, benzene, carbon sulphide and essential oils. Under the action of alkaline bases, turpethin is transformed into turpethic acid, while it gets converted into turpetholic acid, glucose and fructose in presence of hydrochloric acid. Trivit also contains Turpethinic acids- A, B, C, D, and E (Ram Rastogi *et al*., 2002) some ether soluble resin, volatile oil, albumin, starch, lignin salts, ferric oxide, scopoleptin, betulin, lupiol, and beta-sitosterol. Turpethin is mainly responsible for purgative action of Trivit and is an excellent relatively safer substitute for jalap (Nadkarni and Nadkarni, 2007). As a part of our ongoing investigation on Bangladeshi plants for phytochemical, biological and pharmacological properties (Shilpi *et al*., 2005; Uddin *et al*., 2005; Uddin *et al*., 2005), we now report on the acute toxicity and antidiarrhoeal activity of the ethanolic extract of the *Operculina turpethum* in mice.

MATERIALS AND METHODS

Plant material

Operculina turpethum is rare available in Chittagong, Cox's Bazar, Barguna, Jhalukati, Rajshahi, Pabna, Jessore, Kustia, Faridpur and Dhaka districts of Bangladesh. For our study the sample was were collected from the Gazipur near Dhaka and identified by the experts of Bangladesh National Herbarium, Dhaka, where a voucher specimen has been retained. (No. PL-121)

Preparation of ethanol extracts

The *Operculina turpethum* plants are carefully cut with the help of a scissor and separated from other parts. About 600 gm of the *Operculina turpethum* was dried for 15 days without the direct contact of sunrays. When the moisture content of *Operculina turpethum* sample drug was found to be below 5% by loss on drying (LOD) at 100˚C, the *Operculina turpethum* were powdered and weighed (400gm) and then extracted with 95% of ethanol in a Soxhlet's apparatus at an elevated temperature. The extract was concentrated by evaporation under reduced pressure at 40°C using Buchi rotary evaporator to yield a gummy reddish black colored extract (yield App.8.2%)

Drug

Loperamide (Square Pharmaceuticals Limited, Bangladesh)

Animals

Male and female Swiss mice of either sex (20-25 gm body weight) bred in the animal house of the department of pharmacology, Bangladesh Agricultural University, were collected from the animal resources branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B).The animals were housed under standard laboratory conditions (relative humidity 55-60%, r.t 23±2^{o}C and 12 hour light: dark cycle).The animals were fed with standard diet and water *ad libitum.* The institutional animal ethical committee approved the study protocol.

Acute Toxicity Test

An initial test was done to determine the approximate lethal and non-lethal doses of the extract according to Turner (Turner, 1965). Five groups of six mice each were used in the experiments. The extract, at the doses of 2.0, 3.0, 4.0, 6.0 and 10.0 g/kg body weight respectively was administered orally, using intragastric tubes, to the animals as a single dose. The control group was given an equal volume of water. The animals were observed for 24 hours and the number of dead mice was recorded and used in the calculation of the acute toxicity value (LD_{50}). The mice were also observed for other signs of toxicity such as excitation, tremors, twitches, motor coordination, righting reflex, and respiratory changes. A pathologist carried out postmortem examinations of the viscera (stomach, liver, heart, kidney and brain) of the animals.

Antidiarrhoeal activity

Antidiarrhoeal activity of the ethanolic extract of the *Operculina turpethum* was tested using the model castor oil induced diarrhoea in mice (Racusen & Binder, 1979). The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having six mice in each. Group-I was kept as control and received vehicles only (distilled water containing 0.1% Tween-80); group II was treated as 'positive control' and was given the standard drug loperamide at dose of 50 mg/kg of body weight; group III (test group) were treated with the extract of *Operculina turpethum* at dose of 500 mg/kg body weight. Control vehicle, standard drug and the extract were administered orally. 1 hour prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment (5 hours). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for the old ones.

RESULTS

Acute toxicity test

Within six hours of administration of the extract, all the mice that received 10.0 gm/kg of the extract had died. All the animals that received 2.0 gm/kg of the extract survived beyond

the 24 hours of observation. The median acute toxicity value (LD_{50}) of the ethanolic extract of the *Operculina turpethum* determined to be 5.0 gm/kg body weight within 95 % confidence limits (3.45-7.25gm/kg) Table 1. On administration of the extract, no immediate behavioral changes were noted. The mice move and fed normally. After twenty minutes, piloerection was noticed and the animals became restless, some trying to escape through the holes in the cages. The animals did not vomit, neither was there ptosis. The animals that received higher doses went into convulsions and died in hyperextention. Post mortem examination did not reveal any gross abnormality of the brain, the organs of the chest and abdominal cavities. Histopathological examination showed congestion and focal necrosis in the liver and renal tubules (Odida, 1990).

Table 1. Acute toxicity activity of the ethanol extract of *Operculina turpethum* in mice

Number of Mice	Doses of extract gm/kg	Number of mice dead	Mice dead (%)
6	0.0	0	0
6	2.0	0	0
6	3.0	2	33
6	4.0	3	50
6	6.0	5	83
6	10.0	6	100

Table 2. Effect of *Operculina turpethum* ethanolic extracts on castor oil induced diarrhea in mice (latent period)

Treatment	Dose (/kg)	Route of administration	Latent period (h)
Group- I (Control) (1% tween -80)	10 ml	p.o.	1.27 ±112
Group- II (Positive control) Loperamide	50 mg	p.o.	2.18 ±174[a]
Group- III Ethanolic extract	500 mg	p.o.	2.03 ±152[a]

[a]$p<0.001$ vs. control, Student's *t*-test; values are mean ± SEM ($N=6$).

Table 3. Effect of *Operculina turpethum* ethanolic extracts on castor oil induced diarrhea in mice (Number of stools)

Treatment	Dose (/kg)	Route of administration	Period of study (h)	Total number of stool
Group- I (Control) (1% tween-80 solution in water)	10 ml	p.o.	1	2.2 ±307
			2	2.8 ±401
			3	2.6 ±417
			4	3.2 ±477
			5	2.4 ±375
Group- II (Positive control) Loperamide	50 mg	p.o.	1	0.4 ±132
			2	1.6 ±428
			3	2.2 ±269
			4	0.8 ±318[b]
			5	0.4 ±139[b]
Group- III Ethanol extract	500 mg	p.o.	1	1.2 ±441
			2	0.8 ±303[b]
			3	1.5 ±452
			4	1.6 ±499[a]
			5	0.6 ±194[b]

[a]$p<0.05$, [b]$p<0.01$ vs. control, Student's *t*-test; values are mean ± S.E ($N=6$).

Antidiarrhoeal activity

Antidiarrhoeal activity of the ethanolic extract of the *Operculina turpethum* was tested by castor oil induced diarrhoea in mice. The extract caused an increase in latent period (2.03 hour) i.e. delayed the onset of diarrhoeal episode at the dose of 500 mg/kg body weight significantly ($P < 0.05$; $P < 0.01$) which was comparable to the standard drug loperamide at the dose of 50 mg/kg body weight in which the value was 2.18 h ($P < 0.01$) (Table 2). The extract also decreased the frequency of defecation at the same dose where the mean numbers of stool at the 1st, 2nd , 3rd , 4th and 5th h of study were 1.2, 0.8, 1.5, 1.6 and 0.6, respectively and in standard drug the values were 0.4, 1.6, 2.2, 0.8 and 0.4, respectively (Table 3).

DISCUSSION

According to Ghosh (Ghosh, 1984), *Operculina turpethum* can be classified as slightly toxic, since the LD_{50} was found to lie between 0.5 - 5.0 gm/kg. The gram equivalent of the LD_{50} of the extract in an adult man would be 300gm, that is, a plate full of the extract, making it relatively safe. It has been observed that overdose of the *Operculina turpethum* extract is usually non-fatal; the victims tend to suffer self-limiting gastrointestinal disturbances (Anokbonggo *et al*., 1990). Likewise, the extract was administered orally to the test animals. This way the same route used by the traditional healers in treating their patients was used in the test animals. This would make any findings in the mice easily translatable to what would be expected in the human subjects. In any case the extract was not pure enough for parenteral route administration. The viscera of the animals did not show any macroscopic changes that could point to the cause of death. However, since death occurred just after convulsions, it is postulated that the extract killed the mice by some action on the nervous system. Other workers found active principles from the *Operculina turpethum* extract of the plant that appeared to exert their action on the neuromuscular transmission either by blockage of the postjunctional end-plate or by enhancing release of neurotransmitters (Bowen *et al*., 1996). The histological changes that were demonstrated in the

liver and kidney is however significant. However, because of genetic variation in response to drugs by different species, it is difficult to directly translate the results of this study to other animal species or to man. Never the less, in view of the above findings, patients receiving larger doses or under-going prolonged medication with the extracts of the plant, should have renal as well hepatic functions evaluated regularly. These effects are indeed a warning regarding the potential toxicity of the extracts of the plant. Ethanol was used which has a wide range of solubility in both polar and non-polar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness. Acute toxicity studies indicated that *Operculina turpethum* extract can be used safety and should no mortality up to the dose of 2000 mg/kg body weight. So the extract safe for long term administration.Antidiarrhoeal activity of the ethanolic extract of *Operculina turpethum* was tested using the model of castor oil induced diarrhea in mice (Chatterjee, 1993). Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenyl cyclase (Racusen and Binder, 1979) or release prostaglandin (Beubler and Juan, 1979). The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool. On the basis of the result of castor oil induced diarrhoea, it can be concluded that the ethanolic extract of *Operculina turpethum* might possess antidiarrhoeal activity.

Conclusion

It can be concluded the ethanolic extract of *Operculina turpethum* is relatively safe within the normal doses and it might possess antidiarrhoeal activity. However the cerebral mechanism that lead to the death of the mice need to be investigated and determining underlying mechanism of action and to isolate the active principle(s) responsible for antidiarrhoeal activity are also needed.

Acknowledgement

Research work has been performed in Pharmacology Graduate Laboratory, Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202.

RERERENCES

Alam MJ, Alam I, Sharmin SA, Rahman MM, Anisuzzaman M, Alam MF 2010. Micropropagation and antimicrobial activity of *Operculina turpethum* (syn. *Ipomoea turpethum*), an endangered medicinal plant, *Plant Omics Journal*, 3: 40-46.

Anokbonggo, W.W., Odoi-Adome, R. and Oluju, P.M. 1990. Traditional methods of diarrhoeal management in Uganda. *Bulletin of the World Health Organisation*, 68, 359-363

Anonymous. 2001. The Ayurvedic Pharmacopoeia of India. Ministry of health and family welfare, Department of ISM & H, New Delhi

Austin DF. 1982. *Operculina turpethumas* (Convolvulaceae) as a medicinal plant in Asia. *Econ Bot.,* 260:265.

Bapalal Vaidya 2005. Nighantu Aadarsha. Chaukhamba Bharti Publishers, Varanasi.

Beubler E. and Juan H. 1979. Effect of Ricinoleic acid and other Laxatives in Net Water Flux and Prostaglandin E release by the Rat Colon. *J. Pharm. Pharmacol.,* 31, 681-685.

Bowen J.M., Cole R.J., Bedell D. and Schabdach D. 1996. Neuromuscular effects of toxins isolated from southern prickly ash (Zanthoxylum) bark. *Am. J. Vet. Res*., 57 (8): 1239-44

Chatterjee TK. 1993. Handbook of laboratory Mice and Rats. 1st edition, pp. 133-139. Jadavpur University, India.

Deeaph G, Malti G. 1994. Indian Drugs 31: 294.

Fauci AS, Bravnwold E, Isselpacker K, Wilson JD, kasper DL, Hauser SL. and Longo DL. 1993. Harrison's Principles of Internal Medicine. New York, McGraw Hill Company. Vol. (1): 236 -242.

Ghosh, M.N. 1984. Fundamentals of Experimental Pharmacology, 2nd Edition. Scientific Book Agency, Culcutta, 1984; 154-157.

Hardman JG. and Limberd LE. 1992. The Pharmacological basis of therapeutics. In: Goodman and Gilman's (Eds), 10th edition, MacGraw Hill, New York. pp. 914- 931.

Jahan SN. and Sujatha N. 2010. Role of Uttara Vasti with Trivrit and Lasuna oil in the management of primary dysmenorrhea. Ayu 2010, 31(2):228–231

Jain NK. and Saxena VK 1987. Acta Gene. *Indica Chem*., 13: 171.

Kumar SV, Sujatha C, Syamala J, Nagasudha B, Mishra SH 2006. Protective effect of root extract of *Operculina turpethum* Linn against paracetamol induced hepatotoxicity in rats. *Ind J Pharm Sci.,* 68(1):32–35.

Nadkarni KM. and Nadkarni AK 2007. Indian Materia Medica. Bombay Popular Mumbai.

Odida M. 1990. Personal communication.

Racusen LC. and Binder HJ. 1979. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. *J. Clin. Invest*., 63 , 743-749

Ram Rastogi BN, Mehrotra, Sinha S, Shrivastava M, Bhushan B. 2002. Compendium of Indian Medicinal Plants. CDRI Lakhnow and Natrional Institute of Science Communication, New Delhi.

Ram Rastogi BN. 2006. Mehrotra, Shradha Sinha, Pushpa Pant and Renu Sheth, Compendium of Indian Medicinal Plants. CDRI Lakhnow and National Institute of Science Communication, New Delhi.

Sharma PV. and Vidnyana D 2006. Chaukhamba Bharti. Varanasi

Shilpi JA, Ray PK, Sarder MM. and Uddin SJ 2005. Analgesic activity of Amorphophallus campanulatus tuber. *Fitoterapia*, 76: 367-369.

Srikantha Murty KR 2008. Bhavprakasha of Bhavmishra. Jaikrishna Das Ayurveda Series, Varanasi.

Turner, R.A. 1965. Screening Methods in Pharmacology. Academic Press, London.

Uddin SJ, Shilpi JA, Alam SMS, Alamgir M, Rahman MT, *et al*. 2005. Antidiarrhoeal activityof the methanolic extract of the barks of Xylocarpus molucensis in caster oil and magnesium sulphate- induced diarrhea models in mice. *J Ethnopharmacol.,* 101: 139-143.

Uddin SJ, Shilpi JA, Barua J. and Rouf R. 2005. Antinociceptive activity of Ceriops decandra leaf and pneumatophore. *Fitoterapia,* 76: 261-263.

****