Print ISSN 2319-2003 | Online ISSN 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: 10.18203/2319-2003.ijbcp20150015

Research Article

Analgesic and antioxidant activities of the methanolic extract of *Operculina turpethum* leaves in mice

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Received: 19 March 2015
Accepted: 24 April 2015

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ABSTRACT

Background: *Operculina turpethum* Linn. (*Convolvulaceae*) is commonly called "Trivrit" or "India jalap" in English. It is a perennial herbaceous plant with purplish stem and milky-white exudates. This study was aimed at evaluation of the analgesic and antioxidant effects of the methanolic extract of *O. turpethum* leaves in mice.
Methods: The acute oral toxicity of the extract was evaluated using up and down method. The analgesic effects were assessed using acetic acid-induced abdominal writhing reflex and tail flick methods, while the antioxidant activity (AA) was assayed using photometric 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging assay method.
Results: The extract produced a concentration-dependent increase in the AA with inhibitory concentration 50% >400 µg/ml. The extract (100, 200 and 400 mg/kg) and aspirin (100 mg/kg) produced a significant ($p<0.05$) dose-dependent reduction in the number of abdominal constriction induced by intraperitoneal injection of acetic acid in treated mice when compared to the distilled water treated mice. The extract (100, 200, and 400 mg/kg) and pentazocine (3 mg/kg) caused a significant ($p<0.05$) dose-dependent increase in the pain reaction time in the treated mice groups, when compared to the distilled water treated groups.
Conclusion: The study showed that *O. turpethum* possesses analgesic and antioxidant properties and confirmed the folkloric use of *O. turpethum* leaves in the traditional pain management.

Keywords: *Operculina turpethum*, Analgesic, Antioxidant, Acetic acids, Pentazocine, Tail flick response

INTRODUCTION

Pain is an unpleasant sensory or emotional experience often caused by intense or excruciating stimuli (thermal, mechanical or chemical) that may result in actual or potential tissue damage.[1] Pain is a major symptom of many disease conditions and can significantly affect the person's quality of life and functioning.[2] About 59% of adult populations worldwide suffer pain.[3,4] Pain usually resolve following removal of the stimulus and healing of the damaged body tissue; but sometime pain persist despite removal of the stimulus and healing of the body and may also arise in the absence of any detectable damage, stimulus or disease.[5] In orthodox medicine, pain is managed by the use of analgesics and anesthetic drugs. The analgesic drugs are either opioids or non-opioid analgesics. The non-opioid, non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used. In underdeveloped countries of the world, the use of herbal medicine in pain management is gaining wide acceptance due to high cost and side effects of orthodox drugs; perceived low side effects, easy access and cheapness of herbal preparations.[6] Herbs used in pain management include

Bridelia micrantha, *Costus afer*, *Asparagus pubescens*, *Jatropha curcas*, *Sphaeranthus senegalensis*.[7-10]

Operculina turpethum Linn. (*Convolvulaceae*) is commonly called "Trivrit" or "India jalap" in English. It is a perennial herbaceous plant with purplish stem and milky-white exudates. It is pantropical in distribution.[11] The leaves are ovate or oblong with minutely reticulated veins. In folkloric medicine, its roots, stems, and leaves are used in wide range of ailments including gastrointestinal disorders, anemia, asthma, obesity, paralysis, and pain.[12-14] Previous reports have shown that *O. turpethum* possesses hepatoprotective, antiulcer, antidiabetic, and anti-inflammatory activities.[13,15,16] This study was aimed at the evaluation of the analgesic and antioxidant activities of the methanolic extract of *O. turpethum* in mice.

METHODS

Collection and identification of the plant material

Fresh leaves of *O. turpethum* were collected from Ozuitem in Bende Local Government Area of Abia State, South Eastern Nigeria and were confirmed as *O. turpethum* by a plant taxonomist, Dr. M. C Dike of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike. Sample cataloged MOUAU/VPP/2014/011 was deposited in the Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health and Production, College of Veterinary Medicine, Micheal Okpara.

Extraction of plant material University of Agriculture, Herbarium

The leaves of *O. turpethum* were dried at room temperature on a laboratory bench, and were ground to a coarse powder using manual grinder (Corona, China). The powdered material was weighed using an electronic balance. The extract was prepared using cold maceration method. The plant material (150 g) was first macerated with hexane and then the residue was macerated with chloroform and 70% methanol successively for 48 hrs with intermittent shaking every 3 hrs. The respective extracts were filtered with Whatmann No. 1 filter paper and were dried in a hot air oven at 40°C. The *O. turpethum* extract (OTE) was stored in a refrigerator at 4°C until time of use. The percentage yields (w/w) of the extracts were calculated using the formula below:

$$\frac{\text{Weight of extracted material}}{\text{Weight of starting material}} \times \frac{100}{1}$$

Animals

A total of 60 mature mice of both sexes weighing 25-34 g were obtained from the animal unit of Department of Veterinary Parasitology, Michael Okpara University of Agriculture, Umudike. They were housed in aluminum cages under natural light and darkness cycle. Clean drinking water was provided while they were fed with growers (Vital feed® Nigeria) feed *adlibitum* except where fasting was necessary. Ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea[17] and the experimental protocol was approved by Michael Okpara University of Agriculture Umudike Ethical Committee.

Determination of in vitro antioxidant activities of OTE using 2,2-diphenyl-1-picryllhydrazyl (DPPH) photometric assay

The free radical scavenging activity of OTE was analyzed by the DPPH assay using a spectrophotometer. The test extract (2 ml) at different concentrations (25, 50, 100, 200, and 400 µg/ml) each were mixed with 1 ml of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 mins of incubation in the dark at room temperature. The concentrations were prepared in triplicates and the percentage antioxidant activity (AA) was calculated as follows:

% AA = 100-([(absorbance of sample−absorbance of blank)×100]/absorbance of control)

A volume of 1 ml of methanol plus 2.0 ml of the extract was used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard.[18]

Oral acute toxicity study

The oral acute toxicity study of OTE was determined using "up and down" method as described by OECD.[19]

Effect of OTE on acetic acid-induced abdominal writhing in mice

The method of Vale et al.[20] as modified by Onoja et al.[9] was used. Five groups of mice consisting of six mice each were fasted for 12 hrs but given adequate amount of water. Group A mice received distilled water (10 ml/kg) and served as negative control. Group B mice received aspirin (100 mg/kg) orally and served as positive control, while Groups C-E mice received 50, 100, 200 mg/kg of OTE by oral administration, respectively. 45 mins later, the mice received 10 ml/kg of 0.7% acetic acid intraperitoneally. The number of writhing or abdominal stretches produced in each mouse was counted for 30 mins.

Effects of OTE on tail flick response in mice

The experiment was carried out by measuring tail withdrawal time from hot water as described by Adzu et al.[7] 30 mice were randomly divided into five groups (A-E) of six mice each and fasted for 12 hrs. The mice were treated as follows; Group A

served as negative control and received 10 ml/kg distilled water *per os*, Group B served as positive control and received pentazocine (3 mg/kg) intraperitoneally while Group C-E received OTE (50, 100, and 200 mg/kg, respectively) *per os*. One-hr post drug treatment about 2 cm of the tail of each mouse was dipped into a water bath containing warm water maintained at a temperature of 50±1°C. The time taken for the mouse to flick the tail known as the pain reaction time (PRT) was recorded for all the mice.

Statistical analysis

Data obtained were presented as mean±standard error of mean and analyzed using one-way analysis of variance. The variant mean were separated by least significant difference of the different groups. Significance was accepted at the level of $p<0.05$.

RESULTS

Yield of the extract

The extraction of the plant material yielded 7.33% of dark brown oily extract.

Oral acute toxicity study

Oral acute toxicity study showed that the lethal does 50% of the extract is >1500 mg/kg but <2000 mg/kg.

Effects of OTE on DPPH radical scavenging assay

The extract produced a concentration-dependent increase in the AA. The antioxidant activities of OTE were significantly lower when compared to ascorbic acid (Figure 1).

Acetic acid induced abdominal constriction

The extract (100, 200, and 400 mg/kg) and aspirin (100 mg/kg) produced a significant ($p<0.05$) dose-dependent reduction in the number of abdominal constriction induced by intraperitoneal injection of acetic acid in treated mice when compared to the distilled water treated mice. The effects of the extract 200 and 400 mg/kg (88.89% and 91.36%, respectively) on the treated groups were comparable to the effect of aspirin 100 mg/kg (77.22%) on the treated group (Table 1).

Tail flick response

The results of tail flick response are shown in Table 2. The extract (100, 200, and 400 mg/kg) and pentazocine (3 mg/kg) caused a significant ($p<0.05$) dose-dependent increase in the PRT in the treated mice groups, when compared to the distilled water treated groups.

Table 1: Effects of OTE on acetic acid induced writhing reflex in mice.

Treatment	Mean number of writhing±SEM	% inhibition
Distilled water 10 ml/kg	135.00±9.00	-
Aspirin 100 mg/kg	30.75±0.62*	77.22
OTE 100 mg/kg	77.67±0.56*	42.47
OTE 200 mg/kg	15.00±0.73*	88.89
OTE 400 mg/kg	11.67±0.56*	91.36

*$p<0.05$ when compare to the distilled water. OTE: *Operculina turpethum* extract, SEM: Standard error of mean

Table 2: Effects of OTE on tail flick response in mice.

Treatment	Mean PRT±SEM (seconds)	% inhibition of pain
Distilled water 10 ml/kg	2.67±0.30	-
Pentazocine 3 mg/kg	4.13±0.41*	54.68
OTE 100 mg/kg	3.49±0.24*	30.71
OTE 200 mg/kg	3.55±0.32*	32.96
OTE 400 mg/kg	3.70±0.46*	38.58

*$p<0.05$ when compare to the distilled water. OTE: *Operculina turpethum* extract, SEM: Standard error of mean, PRT: Pain reaction time

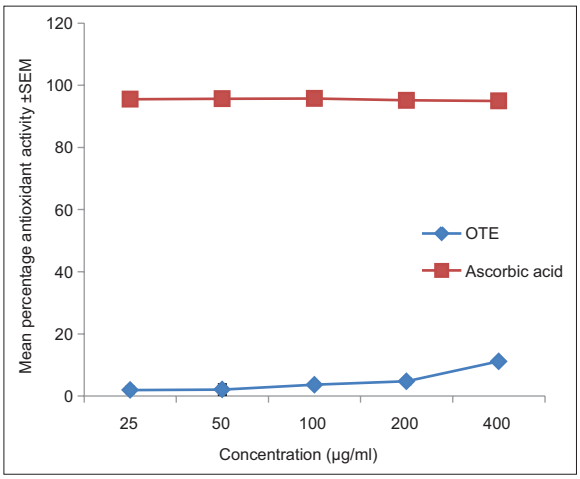

Figure 1: Effect of *Operculina turpethum* extract on 2, 2-diphenyl-1-picrylhydrazyl radical scavenging.

DISCUSSION

This study evaluated the analgesic and antioxidant effects of the methanolic extract of *O. turpethum* leaves in mice. The analgesic effects were assessed using acetic acid-induced abdominal writhing reflex and tail flick methods, while the AA was assayed using photometric DPPH free radical scavenging assay method. The extract produced strong dose-dependent analgesic effect against peripheral pain and weak analgesic effect against deep pain.

The acetic acid-induced abdominal writhing reflex was used as a peripheral pain model. Acetic acid, a commonly used model for assessing the anti-nociceptive effects of drugs, causes an increase in peritoneal fluid of prostaglandin (PG) E2 and PGF2α involving in part, peritoneal pain receptor.[7] The comparable effects of the extract (200 and 400 mg/kg) and aspirin (100 mg/kg) (Table 1) is a clear indication that the extract may have a similar mechanism of action with aspirin. Aspirin is a weak acid and NSAID, which is effective against mild or moderate pain, especially that arising from inflammation or damaged tissue.[21] Aspirin peripherally decrease the production of PGs that sensitize the nociceptors to inflammatory mediators such as bradykinin, serotonin, and cytokines and are therefore effective in conditions associated with increased local PG synthesis.[22] Aspirin reduces PG production through irreversible inhibition of cyclooxygenase (COX-1 and COX-2) activities and thus its duration of action is dependent on the turnover rate of COX in the target tissues.[22]

The tail flick response is a deep pain model.[23] It is mostly used to screen for the effects of the extract on the central nervous system (CNS). The extract significantly ($p<0.05$) increased the PRT of the treated mice to thermal stimulus in a dose-dependent manner when compared to the distilled water treated mice. This suggests that the extract may have increased the pain threshold in CNS of the treated mice.[24] The extract demonstrated a weak CNS involvement when compared to the effects of pentazocine (3 mg/kg). Pentazocine is a synthetic mixed agonist-antagonist with analgesic activities similar to those of morphine.[21] The result of the anti-nociceptive effects of *O. turpethum* leaves is in agreement with the report of Prabhavathi et al.[25]

The extract demonstrated weak AA with an inhibitory concentration of >400 µg/ml. The AA may be mediated by the phytochemical constituents. The AA would be of help in the amelioration of stress that may be induced by pain sensation.[26]

In conclusion, the study has shown that *O. turpethum* leaves possess analgesic and antioxidant properties. The extract is more active against peripheral pain than deep pain and may be acting through the inhibition of COX activities and PGs production. This study confirms the pharmacological basis for the use of the leaves of *O. turpethum* in traditional management of pain. Further work is required for the isolation and characterization of the active analgesic compound.

ACKNOWLEDGMENTS

The authors are very grateful to Dr M. C. Dike of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike for the authentication of the plant material.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The experimental protocol was approved by Michael Okpara University of Agriculture Umudike Ethical Committee

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doi: 10.18203/2319-2003.ijbcp20150015
Cite this article as: Ezeja MI, Onoja SO, Omeh YN, Chibiko CA. Analgesic and antioxidant activities of the methanolic extract of *Operculina turpethum* leaves in mice. Int J Basic Clin Pharmacol 2015;4:453-7.