Phytochemical Screening and Evaluation of Analgesic Activity of *Oroxylum indicum*

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We aimed to study phytochemical screening and analgesic activity of ethanol extract of *Oroxylum indicum*. The dried powder of the barks of the plant was extracted with 95% ethanol and was subjected to various phytochemical tests to ascertain the principle constituents contained in the extract. The result revealed the presence of alkaloids, flavonoids, tannins, glycosides in the ethanol extract of *Oroxylum indicum*. The extract was screened for analgesic activity by using hot plate, acetic acid-induced writhing and formalin test. The ethanol extract of the plant at two different doses (250 and 500 mg/kg) showed significant (P<0.05) analgesic effect in all test methods (hot plate, acetic acid-induced writhing and formalin). The analgesic activity was compared with a standard drug (ketorolac at 10 mg/kg). Based on the present findings and previous literature review it can be concluded that flavonoids and tannins might be responsible for the analgesic activity. We suggest that ethanol extract of *Oroxylum indicum* might have potential chemical constituents that could be used in the future for the development of novel analgesic agent.

Key words: *Oroxylum indicum,* phytochemical, analgesic activity, ethanol extract

A large number of modern pharmacological agents have been isolated from natural sources. The idea of a novel therapeutic agent comes from nature for thousands of years. Many therapeutic agents have identified from the natural origin, several according to their use in traditional medicine[1]. It has been reported that the original source of many important pharmaceuticals in current use have been plants used by indigenous people[2]. About 60% of the total global population remains dependent on traditional medicine and medicinal plants for provision of their healthcare needs[3]. Healthcare providers need diverse therapeutic agents to treat the patient including the analgesic compounds. Phytochemicals produced by the plants such as *Oroxylum indicum vent.* may have analgesic activity allowing these plants to be studied and used for the development of new analgesic drugs.

O. indicum is a well-known medicinal plant in Asia. The root skin, bark and twigs, stems, leaves, flowers, fruit and seeds have all been used to treat a great variety of human ailments for thousands of years[4]. The leaf of *O. indicum* contains chrysin, baicalein[4], while seed contains tetuin, the 6-glucoside of baicalein[4]. *Flavonoids of O. indicum might be responsible* for antiinflammatory[5] and antiallergy activity[6].

Analgesic activity of *O. indicum* is also observed in previous studies. Zaveri *et al.* in 2010 used n-butanol to extract the root bark of *O. indicum*. The authors report that n-butanol fraction (100 mg/kg) significantly reduces the writhing or abdominal constrictions in mouse[7]. Asaduzzaman *et al.*, in 2011 use 3 organic solvents, namely methanol, ethyl acetate and chloroform to extract the stem bark of *O. indicum* and compared the activity with aminopyrine. They have concluded that only methanol extracts (50 ml/kg) shows analgesic activity in writhing test[8].

Previous studies have never been used ethanol to extract the constituents of *Oroxylum indicum* bark. Therefore, we decided to use ethanol. The aim was to investigate the phytochemical components and evaluate the analgesic activity of *O. indicum* by hot plate, acetic acid-induced writhing and formalin test in mice.

Oroxylum Indicum vent. was collected from Jahangirnagar University, Dhaka, Bangaladesh in January, 2011. The plant is found in tropical countries, such as Bangladesh, India, Japan, China, Sri Lanka and Malaysia[4]. The bark portion of the

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plant was collected for study. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh. They were first sun dried and then ground into coarse powder in a grinder. The powder was kept dry until use by storing it in an air tight container. The whole powders were extracted by cold extraction with the solvent ethanol and kept for a period of 3 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then these were filtered through whatman filter paper. The filtrate (ethanol extract) obtained was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68º temperature. It rendered a gummy concentrate of yellowish color. The gummy concentrate was designated as crude extract which was dried by freeze drier and the gummy yield preserved at 4º.

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, saponins, tannin, gums, reducing sugar and terpenoids were carried out for all the extracts by the method described by Harborne and Sazada[9,10]. Phytochemical screening of the extract was performed using the following protocols, reagents and chemicals. Alkaloids were detected using the Dragendroff's test. Ethanol extract of the plant (2 ml) and dilute hydrochloric acid (0.2 ml) were taken in a test tube. After adding 1 ml of Dragendroff's reagent, orange brown precipitate indicated the presence of alkaloids. Flavonoids were detected by adding a few drops of concentrated hydrochloric acid to a small amount of extract solution. Immediate appearance of a red color indicated the presence of flavonoids. Tannins were detected using the ferric chloride test. Ethanol extract (0.5 g) was dissolved in distilled water (5 to 10 ml) and filtered. A few drops of 5% ferric chloride solution were added to the filtrate. A greenish black precipitate was formed which confirmed the presence of tannins. Saponins were identified by diluting the plant extract (1 ml) with distilled water (20 ml) and shaking in a graduated cylinder for 15 min. Layer of foam (approximately 1 cm) indicated the presence of saponins. Salkowski test was used to detect terpenoids. Extract (5 ml) was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids. Molisch test was performed for detecting the existence of gum in the sample. Extract (5 ml) was mixed with molisch's reagent and sulphuric acid. Appearance of red violet ring at the junction of two liquids indicated the presence of gums. Fehling's test was used o detect the presence of reducing sugars. In a test tube 1 ml of Fehling's A and 1 ml of Fehlings's B solution were added. These mixed solutions were boiled for a min. Then equal amount (2 ml) of test solution was added. Brick red precipitate was observed which confirmed the presence of reducing sugar.

Swiss albino mice (12 male and 12 female), group I was designated as control (n=6), group II was designated as ketorolac (n=6), group III was designated as *O. indicum* 250 mg/kg (n=6), and group IV was designated as *O. indicum* 500 mg/kg (n=6), weighing 25-30 g bred in International Center for Diarrheal Diseases and Research, Bangladesh (ICDDR, B) were kept in the animal house of the Department of Pharmaceutical science, North South University. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2°, and 12 h light dark cycle. The animals were fed with standard diet from ICDDRB and had free access to filtered water.

The hot plate test method demonstrates a noxious stimulation in mice[11,12]. Ketorolac was used as a known standard antiinflammatory drug while distilled water was used as a negative control. Ketorolac, distilled water and ethanol extract at 250 and 500 mg/kg[4] given orally 30 min after hot plate induction. If the sample possesses any analgesic activity, then the mouse that was fed the sample will show responses after a prolonged period of time compared to the sample has no activity. A 600 ml test beaker was placed on thermostat hot plate. The temperature was regulated that to 50±1°. Each mouse was placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced nociceptive pain stimulus. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60, 120, 180 and 240 min after oral administration of the samples.

Acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study ketorolac was used to

serve the purpose. The plant extract was administered orally in two different doses (250 and 500 mg/kg) to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 min prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1 ml/10 g). Animals were kept individually under glass jar for observation. Each mouse of all groups were observed individually for counting the number of writhing they made in 10 min commencing just 5 min after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while ketorolac (10 mg/kg) was used as a reference known antiinflammatory drug as a positive control.

Twenty microlitres of 5% formalin was injected subcutaneously into the right hind paw of mice to induce inflammation. The number of licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after injection (early phase) and 20–30 min after formalin injection (late phase). Ethanol extract of *O. indicum* (250 and 500 mg/kg, p.o.) was administered 30 min before formalin injection. Ketorolac (10 mg/kg, p.o.) was administered 30 min before formalin injection. The control group received the same volume of distilled water by oral administration.

The results of statistical analysis for animal experiment were expressed as mean±SEM and were evaluated by one-way ANOVA followed by Dunnet's multiple comparisons. Formalin-induced pain inhibition between the early and late phase were analyzed by independent sample *t*-test. The results obtained were compared with the control group. The $P<0.05$ were considered to be statistically significant. All the statistical tests were carried out using SPSS statistical software.

Preliminary phytochemical screening of ethanol extract of *Oroxylum indicum* revealed the presence of various bioactive components such as alkaloid, flavonoides, terpenoids and tannins. The result of phytochemical test has been summarized in Table 1.

Ethanol extract and ketorolac at the assayed doses results in a significant ($P<0.05$) inhibition on the writhing responses induced by hot plate when compared to the control (distilled water). The results of hot plate test indicated the analgesic activity of the ethanol extract of *O. indicum* as presented in Table 2.

The results of acetic acid-induced writhing responses also indicated analgesic activity of the ethanol extracts of *O. indicum* that were presented in fig. 1. Ethanol extract and ketorolac at the assayed doses produced a significant ($P<0.05$) inhibition on the writhing responses induced by acetic acid when compared to the control.

Ethanol extract of *O. indicum* demonstrated a promising analgesic effect in late phase of the formalin-induced pain. In the early phase, there were no significant inhibitions at the doses of 250 and 500 mg/kg ethanol extract of *O. indicum* compared to the control group. In the late phase, the doses of both 250 and 500 mg/kg significantly reduced the nociception similar to 10 mg/kg ketorolac (Table 3).

TABLE 1: PHYTOCHEMICAL TEST OF ETHANOL EXTRACT OF *OROXYLUM INDICUM*

Test	Tannins	Flavonoids	Terpenoids	Gums and Carbohydrates	Alkaloids	Reducing sugars
Results	+++	+++	+++	+	++	+

'+++' indicates presence in high concentrations; '++' indicates presence in moderate concentrations; '+' indicates presence in trace concentration

TABLE 2: EFFECT OF ETHANOL EXTRACT OF *OROXYLUM INDICUM* ON HOT-PLATE TEST

Treatment with dose	Reaction time at different time intervals (in sec)					
	0 h	½ h	1 h	2 h	3 h	4 h
Control	12.36±1.23	10.74±0.89	9.2±0.67	8.14±0.61	6.67±0.56	6.1±0.51
Ketorolac	9.4±0.65	12.2±0.45* (29.78)	13.7±0.4* (45.74)	15.28±0.51* (62.55)	17.1±0.19* (81.91)	14.4±0.29* (53.19)
O. indicum (250 mg/kg)	8.72±0.54	11.66±1.25 (33.71)	13.18±1.37* (38.89)	14.08±1.41* (61.46)	15.52±0.81* (77.98)	12.1±0.61* (38.76)
O. indicum (500 mg/kg)	8.02±1.1	10.38±0.71* (29.42)	13.38±1.49* (66.83)	15.44±0.8* (62.51)	15.82±0.66* (67.11)	11.1±0.61* (38.40)

Data is represented as mean value±SEM ad analyzed separately by one-way ANOVA followed by Dunnett's *t* test. Significance represented as *$P<0.05$ as compared to control. *N*=6 Mice. The values in parentheses indicate percent increase in reaction time

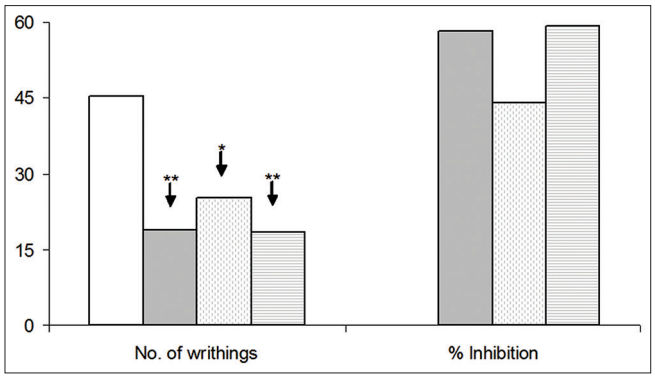

Fig. 1: Effect of ethanol extract of *O. indicum* on acetic acid-induced writhing.
Total number of writhing and percentage of inhibition are represented in horizontal axis. Left side: *O. Indicum* (500 mg/kg) shows minimum number of writhing, followed by ketorolac and *O. Indicum* (250 mg/kg). Control group showed highest number of writhing. Right side: The inhibition of writhing was higher in *O. Indicum* (500 mg/kg) group followed by ketorolac and *O. Indicum* (250 mg/kg). *n*=6 Mice. □ Control, ▩ Ketorolac, ▣ *O. Indicum* (250 mg/kg), ■ *O. Indicum* (500 mg/kg).

The present study demonstrated analgesic activity of ethanol extract of *Oroxylum indicum* bark in hot plate and formalin and acetic acid-induced writhing model in mice. Hot plate test method is one of the most common tests for evaluating the analgesic efficacy. The paws of mice are very sensitive to heat at temperature which does not damage the skin. Responses found in this test are jumping, withdrawal of the paws and licking of the paws[13]. Ethanol extracts of *O. indicum* at the dose levels of 250 and 500 mg/kg exhibited potent analgesic activity in hot plate method. The mouse that was fed the ethanol extract showed prolonged responses time compared to the control. This prolonged reaction time indicates the analgesic activity of the ethanol extract. Analgesic activity was observed even at low dose of 250 mg/kg and in the first hour during test period.

In the acetic acid-induced writhing model, the ethanol extract of *O. indicum* also showed a potent analgesic effect. In this abdominal writhing (or visceral pain model) model, arachidonic acid and prostaglandin biosynthesis may play a role in the nociceptive mechanism. These types of response are caused by peritoneal mast cells acid sensing ion channels and the prostaglandin[14]. Component of ethanol extract of *O. indicum* such as flavonoids might be responsible for this action. The mechanism of analgesic action was reported previously. Annegowda *et al.* in 2010 reported that flavonoids show analgesic action by enhancing the endogenous serotonin level or

TABLE 3: EFFECT OF ETHANOL EXTRACT OF *OROXYLUM INDICUM* ON FORMALIN TEST

Treatment group with dose	0-5 min (early phase)	% Inhibition	20-30 min (late phase)	% Inhibition
Control	25.25±0.47	-	16.25±0.75	-
Ketorolac	12.75±0.75	49.50	4.00±0.40*	75.38
O. Indicum (250mg/kg)	16.25±1.18	35.64	9.25±0.95*	43.07
O. Indicum (500mg/kg)	16.75±0.47	33.67	6.25±1.31*	61.53

Data is represented as Mean value±SEM ad analyzed separately by one-way ANOVA followed by Dunnett's *t* test. Significance represented as *P<0.05 as compared to control. N=6 Mice

interact with 5-HT2A and 5-HT3 receptors[15]. Acetic acid-induced writhing has also been associated with increased prostaglandin level (PGE2, PGF2α) in peritoneal fluids as well as lipoxygenase products[15,16]. Elevated prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability. Ethanol extract of *O. indicum* possibly inhibit the synthesis of the arachidonic acid metabolite and thereby shows analgesic action.

During the formalin test the response time of the animals spends licking the injected paw were measured. Two distinct periods of high licking activity was identified, an early phase lasting the first 5 min and a late phase lasting from 20 to 30 min after the injection of formalin. The results demonstrate that two phases in the formalin test may have different nociceptive mechanisms. It is suggested that early phase is due to a direct effect on nociceptors and prostaglandins do not play an important role during this phase. The late phase seems to be an inflammatory response with inflammatory pain can be inhibited by antiinflammatory drugs.

In our study, the ethanol extracts of the *O. indicum* showed significant analgesic effect. Previous studies suggest that flavonoid have analgesic activity. Therefore, based on our present findings and previous literature report it may be concluded that ethanol extracts of *O. indicum* may have potential component for further investigations and uses in practice. Future study may include isolation of the lead compound and establish the chemical structure as well. Identification of lead compound would be helpful for investigating of analgesic activity of specific component that is present in *O. indicum* in animal model.

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