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EVALUATION OF *IN VIVO* CENTRAL ANALGESIC ACTIVITY AND PRELIMNARY PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *Terminalia brownii* LEAVES

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ABSTRACT

***Background**: Terminalia brownii is a herbal plant which has been used in traditional medicine in western, eastern and southern Africa to treat some disease conditions. Terminalia brownii Fresen (Combretaceae) locally known as "Abalo" in Amharic and "Weiba" in Tigrigna is a medicinal plant which is traditionally used in western, eastern and southern Africa to treat some disease conditions like malaria, hepatitis and yellow fever. **Aim:** The aim of this study is to evaluate the central analgesic activities of 80% methanolic extract of T.brownii leaves in experimental animals. The study was carried out in Pharmacognosy and Phytochemistry laboratory, Department of Pharmacy, College of Health Sciences, Mekelle University, Mekelle. **Method:** Thirty mice (28– 35g) of both sex were used for the study. Tail flick response method was used to assess the central analgesic activity of the plant extract. The methanolic extract of the leaves of T. brownii at the doses of 200 mg/kg 300 mg/kg and 400 mg/kg was used in the present study. Acute toxicity was carried out in mice to determine safe doses for use. Standard phytochemical screening methods were used to test the presence of phytoactive compounds in the plant. **Results:** The preliminary phytochemical screening of the extract showed the presence of flavonoids, phytosterols, polyphenols, tannins, saponin and coumarins. In oral acute toxicity study, no mortality was observed at a dose as high as 2000 mg/kg. The methanolic leaf extract of Terminalia brownii produced significant analgesic effect in a dose dependent manner and an appreciable analgesic effect was noticed at 400 mg/kg dose. **Conclusion:** The present study reveals that the methanolic leaf extract of T. brownii have potential analgesic activity against heat stimuli in the tested animals characterized by a profound increment in the reaction time of tail flick response when compared to the control group.*

KEY WORDS

Terminalia brownii, Analgesic activity, Tail immersion method

INTRODUTION

Natural products continue to play an important role in the discovery and development of new pharmaceuticals, as clinically useful drugs, as starting materials to produce synthetic drugs, or as lead compounds from which a totally synthetic drug is designed[1,2]. The genus *Terminalia* is the second largest genus in the *Combretaceae*, the family is distributed throughout the tropical and subtropical regions of the world and approximately fifty species of *Terminalia* are naturally distributed throughout western, eastern and southern Africa[3,4].

Terminalia species provide economical, medicinal, spiritual and social benefit. Fruits and barks; are important sources of tannin, as well as gum and resins for glazing pottery[5,6]. Leaves, fruits, bark and roots of species such as *T. mollis* Lawson, *T. ivorensis*, *T. laxiflora* Engl. and Diels, *T. catappa* L. and *T. superba*; are sources of dyes of different colors (black, red, orange, yellow, brown) and used for decorating the walls of houses and buildings with murals, for dyeing clothes, mattings, rattan, spoons and walking sticks[7]. The phloem fibres are chewed and the solution swallowed in the treatment of yellow fever, particularly in children. An extract from

the leaves is used to treat pink-eye in livestock and a medicine from the bark is used in the local treatment of hepatitis[8,9]. It is believed that plants which are rich in a wide variety of secondary metabolites, belonging to chemical classes such as tannins, terpenoids, alkaloids, polyphenols are generally superior in their anti-microbial activities. *Terminalia brownii* Fresen (*Combretaceae*) is a well known plant in Ethiopia and widely used in Ethiopian traditional medicine to treat bacterial, fungal and viral infections[10]. The present study was carried out to evaluate the analgesic activity of *T. brownii* leaves in animal models.

MATERIALS AND METHODS

Plant Collection and Extract Preparation

Fresh leaves of *Terminalia brownii* were collected from "Ende-gebremariam", Northwest Tigray and dried. The dried leaves were then powdered with a mechanical grinder and stored in airtight container. The powdered plant material (500gm) was macerated with 2.5 liter of 80% methanol for 3 days with occasional shaking and filtered. The filtrate was remacerated using 1.5 liters of the same solvent each for the next two consecutive three days and filtered. The macerates were then concentrated in a soxhlet rotary evaporator at 60ºC. The solvent was completely removed under reduced pressure and labelled TBL and used for the present study.

Experimental animals

Thirty swiss albino mice (24– 41g) of both sex equal in number were used for the present study. The animals were grouped and housed in cages with not more than six animals per cages and maintained under standard laboratory condition. They were allowed free access to water and standard livestock pellets. The animals were acclimatized to laboratory condition for ten days and examined to be free of wounds, swellings and infections before the commencement of the experiment. All experimental protocols were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

Phytochemical screening

The phytochemical analysis was performed on the ground (powered) leaf of *T. brownii* for identification of the constituents. The constituents tested for were alkaloid, tannins, saponins, free anthraquinones, o-anthraquinone glycosides, cardiac glycosides, flavonoids, polyphenols, coumarins and phytosterols[11].

Acute toxicity test

The acute toxicity of methanol extract of *T.brownii* leaf was determined in mice according to the method of Hilaly *et al*., 2004 with slight modifications[12]. Mice fasted for 4h were randomly divided into 6 groups of five mice per group. Graded doses of the plant's extract (100, 200, 400, 800, 1600, 2000 mg/kg p.o) and control (3ml/kg distilled water) were separately administered to the mice in each of the groups by means of oral route. All the mice in the groups were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period of time was recorded.

Analgesic Activity

Tail immersion method

Mice were divided into five groups of six animals each. Group 1, 2 and 3 received 200, 300 and 400 mg/kg of TBL respectively through oral route. Group 4 recived 2ml/kg of 0.5% methylcellulose (control). Group 5 received the standard drug pethidine (5mg/kg i.p). The animals were held in position with the whole tail extending out. The tail area (2-3 cm) was immersed in a hot water which was thermostatically maintained at 50 ± 2°C, The withdrawal time of the tail from hot water (in-seconds) was noted as the reaction time or tail-flick latency. The maximum cut off time for immersion was 180 seconds in order to avoid injury of the tail tissues. The initial reading was taken immediately before administration of test and standard drugs and then 30, 60, 90, 120, 150 and 180 minutes following administration. The criterion for analgesia was postdrug latency which was greater than two times the predrug average latency. Tail-flick latency difference (TFLD) or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs. Analgesia TFLD was calculated as follows: Analgesia TFLD = postdrug tail flick latency minus predrug tail flick latency[13-16].

Statistical analysis

Statistical analysis values for analgesic activity were expressed as "mean increase in latency after drug administration ± SEM" in terms of seconds. The significance of difference between means was determined by Student's *t*-test. Values of P<0.05 were considered significant and P<O.01 as highly significant.

RESULTS

Phytochemical screening

Preliminary phytochemicals analysis of TBL in this study revealed the presence of tannins, saponins, flavonoids, polyphenols, coumarins and phytosterols.

Oral acute toxicity study

In the present study, a preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiments rather than to provide complete toxicity data of the extract. The 80% methanolic extract of TBL did not show significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions and coma when compared with the normal control groups and none of the animals died up to a dose of 2000 mg/kg indicating that the LD_{50} is greater than 2000 mg/kg and hence three doses, 200 mg/kg, 300 mg/kg and 400 mg/kg were selected for the analgesic activity study.

Analgesic activity

Tail immersion test method

In tail immersion method the extract considerably increased the animals reaction time to the heat stimuli. Values were found to be significant (p<0.05) and even strongly significant (p<0.01) at reaction time 60, 90, 120 and 180. Pretreatment with TBL at the doses of 200, 300 and 400mg/kg increased the reaction time at a dose dependent level.

Table 1 Effect of TBL on tail immersion reaction time in mice

Treatment	Dose (mg/kg)	Reaction time (sec)						
		0min	30min	60min	90min	120min	150min	180min
Control (methyl cellulose)	2	2.93±0.55	8.6±4.12	7.44±1.13	7.2±1.64	5.66±1.26	5.30±1.2	5.50±1.38
Standard pethidine	10	3.562±0.303	37.30±14.35	45.70±15.96**	26.60±8.33**	17.60±6.38	17.70±7.85	21.10±10.74**
TBL	200	5.67±4.32	27.72±24.37	13.30±8.11**	12.23±8.42	10.66±6.3131	7.76±2.68	5.38±1.26**
TBL	300	7.78±2.49	15.27±14.17	10.03±7.77**	12.64±9.01	15.10±9.24	13.64±8.06	9.98±5.14
TBL	400	6.32±2.33	24.48±18.09	25.88±18.03	27.44±12.61**	23.54±13.77**	18.83±14.38	13.83±10.51

Each data represents the statistical mean latency of analgesic responses (sec) ± S.E.M.

All data were found to be significant at 5% level of significance where P<0.05.

** Treatment groups were compared with control ($P < 0.01$).

DISCUSSION

Plant products are in use for long time in folklore medicine for the cure of different diseases with minimal side effects and comparable efficacy. The plant kingdom has been explored for drugs relieving pain[17]. Accordingly this study was undertaken to evaluate the central analgesic activity of methanol extract from *T.brownii* leaf in experimental animal model by using tail immersion method. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. Analgesic effect against thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomena[18]. In this study the extent of activity shown by the crude extracts are less than that of the standard drug pethidine but many fold more than that of the control group, which justifies its activity.

Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure[19]. The effect of the extract on this pain characteristic model indicates that it might be centrally acting. Pharmacological studies have shown that the

naturally -occurring opioid peptide, endorphin, interacts preferentially with μ receptors, the enkephalins with d receptors and dynorphin with k receptors. Morphine has considerably higher affinity for μ receptors than for other opioid receptors. The opioid antagonist, naloxone, inhibits all opioid receptors, but has highest affinity for μ receptors. All 3 receptors produce analgesia when an opioid binds to them[20]. Hence the mechanism of action of TBL may be due to activation of one of the three opioid receptors specially the μ receptors.

Several secondary metabolites isolated from different medicinal plants have been discovered to posses' inhibition of pro-inflammatory mediators such that reducing pain. The metabolites responsible for this effect are flavonoids, tritepeinoids, sterols (phytosterols), tannins, alkaloids, anthraquinones, coumarins, polyphenolic compounds etc. and in this study the preliminary phytochemical screening revealed the presence of flavonoids, saponins, tannins, coumarins, polyphenols and phytosterols[21]. Therefore, the analgesic activity of the extract is thought to be mediated by the presence of flavonoids, phytosterols, polyphenols, tannins, saponins and coumarins.

CONCLUSION

In conclusion, this study has shown that the methanolic extract from the leaf of *T.brownii* does possess significant analgesic activity in laboratory animals at the doses tested and the results were comparable with the standard drug morphine. It is also suggested that the mechanism of analgesic action of TBL might be associated with activation of one of the three opioid receptors specially μ receptor.

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