
Full Length Research Paper

Analgesic properties of *Tamarindus indica* linn stem bark fractions in albino rats

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The research work was conducted to investigate the phytochemical constituents and analgesic activities of *Tamarindus indica* linn stem bark fractions. The fractions (hexane 13.6%, ethyl acetate 12.1%, methanol 7.9% and water 6.7%) were obtained using sequential fractionation. The analgesic activity of *T. indica* stem bark fractions at dose of 200 mg/kg were evaluated using acetic acid- induced writhing test and tail immersion test in albino rat. All the fractions tested significantly ($P < 0.001$) inhibited acetic acid induced writhing while only methanol and water fractions significantly ($P < 0.05$) prolonged the reaction latency to pain thermally induced in rat by tail immersion test. Phytochemical constituents present in the fractions were found to include Flavonoids, saponins, alkaloids, tannin and glycosides which might be responsible for the observed analgesic activity. The result suggest that *T. indica* linn stem bark fractions possesses effective analgesic activity mediated via peripheral and central mechanisms.

Key words: *Tamarindus indica*, analgesic; writhing, antinociceptive, fractions.

INTRODUCTION

Man from time immemorial has used various substances from animals, plants and minerals sources to alleviate pain. Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in term of such damage (Bonijja, 1979). Many pathological disorders are accompanied with painful conditions. Pain and fever are the most common complaints associated with inflammation. The NSAIDs used in the treatment of pain and inflammatory conditions do not cure and remove the underlying cause of the disease but only modify the response to the disease (Anupama et al., 2012). These drugs are not entirely free of side effects and have their own limitation (Reynold, 1993).

Due to adverse side effect caused by NSAIDs, tolerance and dependence induced by opiates the use of these drugs as analgesic agents have not been

successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs (Kumara, 2001).

Tamarindus indica locally known as 'Tsamiya' in Hausa language belongs to the family Fabaceae. The tree is endemic to tropical Africa, particularly in Sudan; it is also cultivated in Cameroon, Nigeria and Tanzania. Different parts of this plant are used in the indigenous systems of medicine for the treatment of a variety of human ailments (Nikkon et al., 2003; Rahman et al., 2001; Khalid et al., 2010). In Africa, it is used for the treatment of diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Ferrara, 2005). It is applied on inflammations, used as a gargle for sore throat and mixed with salt as a liniment for rheumatism, accelerate

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expulsion, relieve pain, reduce secondary bacterial and promote healing (Fabiya et al., 1993). In Nigeria, particularly in the North western part, *T. indica* stem bark is being used pain relief in the treatment of fracture and dislocation of bone. In view of the traditional claim, *T. indica* stem bark was targeted for investigation to ascertain and document its therapeutic efficacy or otherwise, in treatment of pain and inflammation.

MATERIALS AND METHODS

Drugs

The following chemicals and drugs were used: Acetic acid solution (E-merk England Limited), Aspirin and Diclofenac sodium (Globela Pharma PVT. Ltd, India).

Collection of plant material

The fresh stem bark of *T. indica* was collected from Aliero town, Kebbi State, Nigeria in the month June. The plant specimen was identified taxonomically and authenticated by Dr D. Singh, Department of Biological Science, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.

Preparation of plant extract

The air-dried stem bark of *T. indica* was made in to a coarse powder. The powdered material was macerated using 50% methanol for 24 h. The hydromethanolic extract was filtered through muslin and the filtrate was evaporated to dryness in an oven set at 45°C giving a light brown solid with a yield of 32% with reference to dry starting material. Solutions of the extract were prepared freshly with distilled water and used for phytochemical analysis.

Sequential fractionation

The powdered dried sample of *T. indica* Linn stem bark (200 g) was extracted sequentially with solvents of different polarity (hexane, ethyl acetate, methanol and water using sequential extraction procedure (Bruneton, 1999).

Experimental animals

Albino rats of both sexes weighing 130 – 180 g were used for the study. They were purchased from the animal house of Nigerian Trypanosomiasis Research Institute (NTRI), Kaduna, Nigeria. All the animals were kept in the cage and allowed to acclimatize for two weeks in Biochemistry laboratory, Kebbi State University of Science and Technology, Aliero, before the

commencement of the experiment. The animals were fed with standard diet and water *ad libitum*.

Preliminary phytochemical screening

Phytochemical analysis was performed on the crude extract and fractions of *T. indica* stem bark using standard procedures to identify chemical constituents (Trease and Evans, 1989; Mbatchou and Kosoono, 2012; Sofowora, 1993).

Analgesic activity

Acetic acid- induced abdominal writhing test

This test was performed using the method described by Zakaria et al. (2008) with slight modification. The animals were divided into six groups of five animals each. Group I served as normal control and received distilled water (1 ml/kg, p.o), groups II - V served as treatment groups and were administered with 200 mg/kg of hexane, ethyl acetate, methanol and aqueous fractions of *T. indica* stem bark respectively. Group VI served as reference group and received Diclofenac (20 mg/kg, p.o). Acetic acid (1% v/v) was administered intraperitoneally to all the groups at the dose of 1 ml/kg body weight 30 min after the administration of test compounds. Anti-nociception was recorded by counting the number of writhes after the injection of acetic acid for a period of 10 min. A writhe is indicated by abdominal constriction and full extension of hind limb. Antinociceptive activity was expressed as the percentage reaction or inhibition of the number of abdominal writhing.

$$\% \text{ inhibition} = \frac{\text{Mean No. of writhes (control)} - \text{Mean No. of writhes (test)}}{\text{Mean No. of writhes (control)}} \times 100$$

Tail immersion test

Basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source (water bath thermo-statistically maintained at $55 \pm 1.0^\circ\text{C}$) according to the method described by Kumar and Shankar (2009) with slight modification. The animals were divided into six groups of five animals each. Group I served as normal control and received distilled water (1 ml/kg, p.o), groups II - V served as treatment groups and were administered with 200 mg/kg of hexane, ethyl acetate, methanol and aqueous fractions of *T. indica* stem bark respectively. Group VI served as reference group and received Aspirin (20 mg/kg, p.o). The tail withdrawal from the heat (flicking response) is taken as the end point. A cut off period of 15 s is observed to avoid damage to the tail. The measurements of withdrawal time was conducted at 30,

Table 1. Percentage yields of *T. indica* linn stem bark fractions.

Extractant	Mass extracted (g)	% yield
Hexane	27.1	13.6
Ethyl acetate	24.2	12.1
Methanol	15.8	7.9
Water	13.4	6.7

Table 2. Phytochemical screening of *T. indica* linn stem bark.

Phytochemicals	Crude Extract	Hexane Extract	Ethyl acetate Extract	Methanolic Extract	Aqueous extract
Saponin	++	-	-	++	+
Alkaloids	+++	+++	+++	+++	+++
Flavonoids	-	-	-	-	++
Terpenoids	+++	+++	+++	++	+
Steroids	++	++	+++	-	+
Glycosides	-	++	++	++	++
Phlobatannins	-	-	-	-	-
Anthraquinone	-	+	+	-	-
Cardenolides	++	-	-	+	-
Tannin	+++	+++	+++	+++	+++

KEY: + = slightly present; ++ = moderately present; +++ = highly present; and - = absent.

Table 3. Effect of *T. indica* linn stem bark fractions on acetic acid induced writhing test in albino rats.

Groups	Dose (mg/kg)	Number of Writhing	Percentage Inhibition (%)
Control	Vehicle	45.25±2.29	-
Hexane extract	200	16.75±3.35	62.98
Ethyl acetate extract	200	21.33±2.40	52.81
Methanolic extract	200	20.75±3.90	54.14
Aqueous extract	200	19.00±4.30	58.01
Diclofenac	20	22.75±4.91	49.72

Values are mean ± SEM (n=5) *P < 0.01, Dunnet test as compared to the control.

60, 90 and 120 min after administration of drugs and % inhibition was calculated as

$$\% \text{ inhibition} = \left(\frac{T_1 - T_0}{T_0} \right) \times 100$$

T₁ is post-drug latency and T₀ is predrug latency.

Statistical analysis

Effect on Acetic acid-induced writhing

All the fractions (hexane, ethyl acetate, methanol and aqueous) evoked significant (P < 0.01) and dose-dependent reduction in the number of acetic acid-induced writhes in rats when compared to control. The highest percentage inhibition of abdominal constriction was

The data was expressed as Mean ± SEM of 5 animals. Analysis of variance (ANOVA) followed by Dunnett-test was used to statistically analyze data. P values less than 0.001 (P < 0.01) were considered significant for acetic acid induced test and (P < 0.05) for tail immersion test.

RESULTS

The percentage yields and phytochemical analysis of the crude extract and sequential fractions of *T. indica* are shown in Tables 1 and 2 respectively. observed in hexane fraction (62.98%) and was greater than that of the Diclofenac (49.72%), the standard drug (Table 3).

Effect on tail flick response

Only the aqueous and methanolic fractions of *T. indica* stem bark produced a significant (P < 0.05) increase in

Table 4. Effect of *T. indica* Linn stem bark fractions on tail immersion test in albino rats.

Groups	Dose(mg/kg)	0 min	30 mins	60 mins	90 mins	120 mins
Control	Vehicle	1.24±0.17	1.31±0.14	0.95±0.85	0.89±0.05	0.98±0.08
Hexane	200	1.86±0.25	2.08±0.52	0.86±0.06	1.65±0.44	1.18±0.24
Ethyl acetate	200	1.13±0.22	1.62±0.54	1.33±0.68	1.38±0.15	1.51±0.12
Methanolic	200	1.13±0.52	3.63±0.49 [*]	2.79±0.52 [*]	3.07±0.26 [*]	2.62±0.39 [*]
Aqueous	200	1.16±0.11	4.88±0.86 [*]	3.96±0.43 [*]	4.04±0.63 [*]	2.84±0.18 [*]
Aspirin	20	1.90±0.29	2.67±0.15 [*]	3.27±0.18 [*]	2.72±0.10 [*]	3.27±0.33 [*]

Values are mean ± SEM (n = 5) ^{*}P<0.05 Dunnet test as compared to the control.

the reaction time to tail flick when compared to control. The percentage inhibition of the aqueous and methanolic fraction (200 mg/kg) was comparable to that produced by the standard drug aspirin (20 mg/kg) (Table 4).

DISCUSSION

The antinociceptive activity was evaluated using both chemical and thermal methods of nociception in rat. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia, whereas hot plate and tail flick tests are most sensitive to centrally acting analgesics. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed et al., 2006) via cyclooxygenase (COX), and prostaglandin biosynthesis (Duarte et al., 1988). In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2 α in peritoneal fluids as well as lipooxygenase products (Deraedt et al., 1980). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria et al., 2008). The acetic acid induced writhing method has been found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte et al., 1988; Ferdous et al., 2008). The significant pain reduction of all the plant fractions might be due to the presence of analgesic principles acting within the prostaglandin pathways. The abdominal writhing induced by acetic acid has been reported to be less selective (Collier et al., 1968) and proposed to act indirectly by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics and centrally acting agents (Toma et al., 2003).

Thermal induced nociception indicates narcotic involvement (Besra et al., 1996). Thermal nociceptive tests are more sensitive to opioid μ receptors and non-thermal tests are to opioid κ receptors (Abbott and Young, 1988; Furst et al., 1988). The tail immersion test is considered to be selective for the drugs acting central.

It measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity (Sabina et al., 2009). The aqueous and methanolic fractions of *T. indica* stem bark only, presented a longer latency time than the control group in the tail immersion test. This suggests that the aqueous and methanolic fractions are more effective in alleviating central pain. Opioid analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain (Elisabetsky et al., 1995; Pal et al., 1999). The water and methanol fractions of *T. indica* stem bark exhibited both types of pain inhibition. This result suggests that these fractions may possess NSAID-like and Opioid-like analgesic activities, mediated through both peripheral and central mechanisms (Sabina et al., 2009).

The pharmacological activities of medicinal plants are usually due to their secondary metabolites. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, saponin and tannins. Flavonoids have been reported to play a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana et al., 2001; Rao et al., 1998). There are also reports on the role of tannins in anti-nociceptive activity (Vanu et al., 2006). Besides, alkaloids are well known for their ability to inhibit pain perception (Uche et al., 2008). Therefore, it is assumed that these secondary metabolites may be responsible for the observed analgesic effects.

Conclusion

The results of this study provides evidence for the analgesic effect of *T. indica* stem bark fractions thus supporting the validity of its use in the treatment of painful conditions associated with fractured and dislocated bones. Further work is suggested to isolate active compounds responsible for the analgesic activity and to elucidate the full mechanisms of action involved.

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