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Protective effects of crude and alkaloidal extracts of *Tamarindus indica* against acute inflammation and nociception in rats

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ABSTRACT

Objective: To investigate the anti-inflammatory and antinociceptive effects of total alkaloids extracted from the leaf of *Tamarindus indica* (*T. indica*) in rats.
Methods: Acetic acid-induced pain and egg albumin-induced inflammation were used to inspect the anti-nociceptive and anti-inflammatory effects of the crude and alkaloidal extracts of *T. indica* at doses of 40 and 400 mg/kg, respectively. Sodium diclofenac was used as the control drug.
Results: The percentage yields of crude methanol and alkaloidal extracts of *T. indica* were 2.85% and 0.98%, respectively. Screening of secondary metabolite of the crude extract revealed the presence of saponins, alkaloids, tannins, steroids, phenols and terpenes, while phlobatannins was not detected. The safe dose and LD_{50} were 400 and 750 mg/kg for crude methanol extract, respectively, while the safe dose and LD_{50} of alkaloidal extract were 40 and 57 mg/kg, respectively. The anti-inflammatory and antinociceptive effects of crude methanol extract and alkaloid extract of *T. indica* were significantly ($P < 0.05$) different from those of control rats. The standard drug (sodium diclofenac), crude extract and alkaloidal extract showed percentage inhibition of 89.36%, 53.92% and 81.37% in paw edema, respectively.
Conclusions: The results obtained indicated that the crude and alkaloidal extracts of the plant exhibited significant anti-inflammatory and antinociceptive activities, thus, supporting its folkloric use for the treatment of these conditions.

1. Introduction

Inflammation is a common etiologic factor that contributes to the exasperation of ample variety of disease condition which includes arthritis, asthma and cardiovascular disease[1]. Inflammation is a defence mechanism by host against infections, foreign substances and tissue injury by activation of the cellular immune responses that stimulates various cytokines and nitric oxide which are known as pro-inflammatory mediators[2]. Excessive production of inflammatory mediators could, however, result in a chronic disease condition such as autoimmune disease and rheumatoid arthritis with features of redness, swelling, pain transmitted by peripheral or central nerves[3].

A number of mediators such as eicosanoids, reactive oxygen and nitrogen species, cytokines like tumor necrosis factor-α, interleukin-1β and interleukin-6 have been implicated in the pathogenesis of these inflammatory disorders which provides a plethora of targets for anti-inflammatory molecules[2].

Many drugs have been developed for the treatment of nociception and inflammation. Examples of such drugs include dexamethasone and aspirin (non-steroidal anti-inflammatory drug), opioids and morphine. However, these drugs are allied with undesired adverse effects. Thus, the search for natural products from plants having protective and therapeutic values with minimal side effects is largely becoming the final hope for mankind[4].

The beneficial effects of medicinal properties can be attributed to their active compounds with different chemical and structure configuration. Among them, alkaloids are one of the largest groups, also with a great chemical diversity. In fact, plants are estimated to produce approximately 12000 different alkaloids with wide range of pharmacological properties, including anti-inflamatory and antinociceptive[5,6].

Tamarindus indica Linn. (*T. indica*) (family: Fabaceae) is an important food in the tropics with numerous applications in chemicals, nutritional, textile and pharmaceutical industries[7]. It is indigenous to tropical Africa but it has been naturalized in more

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than 50 countries globally[2]. Tamarind has been characterized by the presence of all amino acids except tryptophan[8]. It also contains several phenolic compounds and other compounds like tartaric acid, mucilage, pectin, uronic acid and triterpenes[9]. The plant is also used traditionally for the treatment of inflammatory disease, abdominal pain, diuretics and gastrointestinal disorders such as constipation[10]. The present study investigated the anti-inflammatory and antinociceptive effects of total alkaloids extracted from the leaf of *T. indica*.

2. Materials and methods

2.1. Plant materials

Fresh leaves of *T. indica* were collected between August and September, 2015 from Maikunkele area of Minna, Northern Nigeria and were authenticated by a botanist at the Department of Biological Sciences, Federal University of Technology, Minna.

2.2. Experimental animals

Swiss adults albino rats weighing between (120.00 ± 3.12) g and (175.00 ± 1.34) g were obtained from the animal house of the Department of Biochemistry, School of Life Sciences, Federal University of Technology Minna, Nigeria. The rodents were housed in standard environmental conditions of 70% relative humidity, (27 ± 20) ℃ of temperature, 12 h night/day light cycles, free water access and pellets.

2.3. Ethical approval

All experimental procedures involving animals were conducted in accordance to Canadian Council on Animal Care Guidelines and Protocol Review and approved by the Federal University of Technology, Minna Committee on ethics for medical and scientific research[11].

2.4. Preparation of extracts

About 40 g air-dried leaves of *T. indica* were pulverized and extracted in the cold with 300 mL methanol. The extract was filtered with Whatman No. 5 filter paper and solvents were removed under reduced pressure in a rotary evaporator. Brown coloured paste obtained was weighed and stored in refrigerator prior to further analysis.

2.5. Screening for secondary metabolites

Standard screening test was used to detect the presence of alkaloids, terpenes, tannins, saponins, phenols, steroids, phlobatannins and flavonoids in the extract[12-14].

2.6. Extraction of alkaloids

The alkaloidal extract of *T. Indica* was obtained according to the methods previously reported[15]. Briefly, 1 000 g of oven dried *T. indica* was extracted with ethanol in a Soxhlet apparatus for 8 h and evaporated under reduced pressure. The pH was adjusted to 1–2 using 2.5% HCl and then filtered, and the filtrate was stored overnight at room temperature followed by pH adjustment to 9.5 using NH_3OH and then extracted with CL_2CH_3. The extract was dried over magnesium sulphate and the solvent was evaporated to yield the crude extract of total alkaloids. After evaporation, the yield of each fraction was calculated and the alkaloid extract of *T. indica* obtained was stored at 4 ℃ until used.

2.7. Safe dose and acute toxicity (LD_{50})

Five groups of four mice each were used and the animals were gavaged extract at doses of 200, 400, 600, 800, 1 200 mg/kg body weight, respectively. Alkaloidal extract of *T. indica* was administered at doses of 20, 30, 40, 50 and 60 mg/kg body weight to the animals in each group. The control group was given normal saline at 20 mL/kg body weight. The mice were observed over a 72 h period for any adverse reactions and mortality was recorded. LD_{50} was determined by the method of Lorke[16].

2.8. Antinociceptive test

Analgesic effect was assessed according to the method described by Nwafor *et al.*[17]. A total of 20 rats were grouped into four of five rats each. Groups A–C were administered with 400 mg/kg body weight of the crude extract, 40 mg/kg body weight of alkaloidal extract and 20 mg/kg body weight of sodium diclofenac, respectively for 60 min before they were challenged with 0.75% v/v acetic acid. Group D (control group) received 20 mL/kg body weight of normal saline. The number of abdominal constrictions induced by acetic acid were counted after 5 min. Observations were made over 10 min and mean value for each group was calculated. Percentage inhibition of abdominal constriction by the plant extracts and sodium diclofenac were determined in relation to the control.

2.9. Anti-inflammatory activity

The anti-inflammatory activity of the extract was tested using egg albumin-induced paw oedema in rats[18]. A total of 16 albino rats were divided into four groups of four animals each. Inflammation was induced by the injection of 0.01 mL egg albumin into the sub-planter surface on the right hind paw 30 min after administering the 400 and 40 mg/kg body weight *i.p.* of crude and alkaloidal extract, respectively. The increase in volume (cm^3) of the hind paw was measured with a LETICA digital plethysmometer at 20 min interval for a period of 2 h. Control rats received an equivalent amount of normal saline with sodium diclofenac (20 mg/kg body weight) served as reference. The percentage inhibition of oedema was calculated for each extract.

2.10. Statistical analysis

Data were presented as mean ± SEM after being subjected by SPSS version 21. Comparisons between different groups were done using ANOVA and Duncan's multiple range test. Values of $P < 0.05$ were considered as statistically significant[19].

3. Results

The percentage yields of *T. indica* methanol crude and alkaloidal extracts were 2.85% and 0.98%, respectively. Phytochemical screening of leaf extract of *T. indica* revealed the presence of saponins, alkaloids, tannins, steroids, phenols, terpenes while phylobatannins was not detected (Table 1). The safe dose and LD_{50} were 400 and 750 mg/kg for crude methanol extract, respectively (Table 2), while the safe dose and LD_{50} of alkaloidal extract were 40 and 57 mg/kg, respectively (Table 3). The standard drug (sodium diclofenac), crude extract and alkaloidal extract showed percentage inhibition of 89.36%, 57.45% and 6.38% in paw oedema, respectively (Table 4), while the percentages of anti-nociceptive activities were 46.08%, 53.92% and 81.37% for crude extract, alkaloidal extract and standard drug, respectively (Table 5).

Table 1
Qualitative phytochemical composition of methanol extract from *T. indica* leaves.

Secondary metabolites	Inference
Alkaloids	+
Teprenes	+
Tannins	+
Saponins	+
Phenols	+
Steroids	+
Phlobatannins	–
Flavonoids	+

+: Present; –: Absent.

Table 2
Acute toxicity profile of crude extract of *T. indica*.

Doses (mg/kg body weight)	Observation	Mortality (%)
100	No noticeable changes	0
200	Rats appeared normal	0
400	Slow stimuli response	0
600	Increased heartbeat, restlessness and mortality	25
800	Tachycardia and mortality	75

Safe dose: 400 mg/kg body weight; LD_{50}: 750 mg/kg body weight.

Table 3
Acute toxicity profile of alkaloidal extract of *T. indica*.

Dose (mg/kg body weight)	Observation	Mortality (%)
20	Rats appeared normal	0
30	No apparent physical changes	0
40	Loss in physical activity and appetite	0
50	Most were unresponsive to stimuli an one mortality	25
60	Hair loss, lacrimation erythema	75

Safe dose: 400 mg/kg; LD_{50}: 750 mg/kg body weight.

Table 4
Anti-inflammatory effect of crude and alkaloidal extracts of *T. indica* in rats.

Groups	Dose (mg/kg)	Time (min)						Paw oedema (%)
		20	49	60	80	100	120	
Normal saline (mL/kg)	20	0.96 ± 0.03	0.85 ± 0.02	0.98 ± 0.03	0.93 ± 0.03	0.92 ± 0.02	0.95 ± 0.01	–
Diclofenac	20	0.12 ± 0.01	0.14 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	89.36
Crude extract	400	0.40 ± 0.01	0.44 ± 0.02	0.40 ± 0.01	0.41 ± 0.01	0.38 ± 0.01	0.41 ± 0.02	57.45
Alkaloidal extract	40	0.88 ± 0.03	0.88 ± 0.01	0.90 ± 0.03	0.91 ± 0.02	0.89 ± 0.02	0.88 ± 0.03	6.38

Values were expressed by mean ± SEM (n = 4) and significant at $P < 0.05$ compared to the control.

Table 5
Effects of crude and alkaloidal extracts of *T. indica* on acetic acid-induced writhing test in rats.

Groups	Dose (mg/kg)	Writhing	Inhibition (%)
Normal saline (mL)	20	40.80 ± 1.02	-
Diclofenac	20	7.60 ± 0.81[a]	81.37
Crude extract	400	22.00 ± 0.71[c]	46.80
Alkaloid extract	40	18.80 ± 0.50[b]	53.92

Values were expressed by mean ± SEM (n = 5) and significant at $P < 0.05$ compared to the control.

4. Discussion

The result of the screening of secondary metabolites of the methanol extract from *T. indica* revealed the presence of flavonoids, saponins, alkaloids, tannins, terpenoids, steroid and absence of phlobatannins. Bioactive compounds such as flavanoids, saponins, alkaloids, terpenes and tannins present in the extract have been reported for analgesic and anti-inflammatory activities[20]. However, the absence of phlobatannins in *T. indica* agrees with previous study that not all phytochemicals are present in all plants and those present vary with the solvent used in the extraction[21].

Acute toxicity studies are used to determine the amounts/concentration of chemicals substances/plants that can produce a detrimental effect on animals upon short-term administration[22]. Result of safe dose and LD_{50} for the crude extracts of *T. indica* indicated that it is safe up to 600 mg/kg body weight in rat which is in compliance with the guidelines of Organisation for Economic Co-operation and Development documented in 2005, while the crude alkaloid showed a safe dose up to 50 mg/kg body weight. Above these concentrations, adverse effects were observed. About 50% mortality was observed at 750 mg/kg body weight while that of crude alkaloid was observed at 57 mg/kg body weight in rats.

Natural products have been established to assuage pains and inflammation in *in vivo* and *in vitro* models. The present study revealed that *T. indica* significantly decreased the number of writhes in test animals. Nociception of rats dosed with 400 and 40 mg/kg body weight of the extracts was significantly reduced since the percentage inhibition obtained was quite comparable with the positive control diclofenac sodium. The result obtained was in line with the work of Adebayo *et al.*[23]. This study showed that alkaloids are known to be the active component to relieve pain which is in accordance with previous study[24]. There are some reports on the role of tannins and flavonoids in antinociceptive activity[3].

The egg albumin-induced right hind paw oedema in rat is sensitive to cycloxygenase inhibitors and is used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibits the cycloxygenase involved in synthesis of prostaglandins[25].

The present study also indicates that methanol leaf extract of *T. indica* significantly produces anti-inflammatory effect probably by inhibiting the release or synthesis of inflammatory mediators, prostaglandins and polypeptide kinins. The percentage inhibition of the crude extract was comparable with that of the standard drug. Previous studies reported that plants rich in triterpenoids, flavonoids and saponins showed anti-inflammatory activity[26].

Although, alkaloids, phenols and tannins have been reported to play a role in the reduction of pain and also function as anti-inflammatory agent. The crude alkaloidal extract of *T. indica* showed no anti-inflammatory activity. Flavonoids are implicated in having anti-inflammatory, anti-pyretic and antioxidant properties[27-29]. Polyphenols and flavonoids have been reported to exert anti-inflammatory activity through the inhibition of activation of nuclear factor-kappa B and down-regulation of the expression of inflammatory enzymes *e.g.* COX-2, 5-LOX and MMP-9[3]. Therefore, the anti-inflammatory activity demonstrated by this plant could be through this mechanism.

The study showed that crude extract of *T. indica* exhibits some degree of inhibition against egg albumin-induced inflammation and alkaloid is probably not the active component. However, flavonoids and other secondary metabolites have been implicated in mediating such activities. Also, the result showed a significant activity in acetic acid-induced nociception in rats thus validating the use of *T. indica* in ethnomedicine for relieving pain.

Conflict of interest statement

The authors report no conflict of interest.

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