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Studies on Analgesic and Anti-Inflammatory Activities of Aerial Parts of *Tephrosia Bracteolata* GUILL. and PERR. (Fabaceae) in Rodents

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background:*Tephrosia bracteolata* is a widespread shrub belonging to the family (Fabaceae) and genus *Tephrosia*. It is traditionally used for treating rheumatic pains, dropsy and stomach ache.

Objectives: In view of the ethnomedicinal claim and the continuous search for new medicinal agents, the phytochemical constituents, analgesic and anti-inflammatory activities of chloroform fraction (CF) of the methanol extract of *Tephrosia bracteolata* in mice and rats was evaluated.

Methods: Preliminary phytochemical screening was conducted using standard method. Analgesic activity of CF (100, 200 and 400 mg/kg body weight orally) was investigated using acetic acid-induced writhing test and thermally induced pain model in mice. Additionally, anti-inflammatory activity was tested by carragenaan-induced paw edema in rats.

Results: Phytochemical screening revealed the presence of alkaloids, triterpenes and flavonoids. The oral LD_{50} of CF was above 2000 mg/kg body weight. CF significantly ($p<0.05$) and dose dependently reduced the number of writhes with percentage inhibition of 47.76 48.41 and 72.6 % at dose of 100, 200 and 400mg/kg respectively. CF also significantly ($p<0.05$) and dose dependently increased the mean reaction time. At dose of 400 mg/kg, CF at 60 and 90 minutes exhibited greater activity when compared to the standard agent pentazocine. CF(200 and 400 mg/kg) at times 3, 4 and 5 hours significantly ($p<0.05$) decreased the paw edema in rats when compare with the ibuprofen treated group.

Conclusions: The chloroform fraction of the methanol crude extract of *Tephrosia bracteolata* possesses analgesic and anti-inflammatory activities.

Keywords: *Tephrosia bracteolata*, Phytochemical screening, Toxicity, Analgesic activity, Anti-inflammatory activity

INTRODUCTION

Medicinal plants have been estimated to be the primary source of health care needs of over 80% of the world population living in developing countries (Gabriel *et al*., 2018). The process of searching useful plants from different records to the development of methods for the industrial production of drugs is termed Ethnopharmacology and it plays an important role in the discovery of new biologically active compounds (Dong., 2013).

Tephrosia bracteolata is a shrub of widespread belonging to the family Fabaceae that grows in uncultivated areas of tropical and warm-temperate regions. There are approximately 400 species included in this genus (Burkill., 1985). Onaolapo *et al*., 2004 reported the toxicity and anti-pyretic studies of the crude methanolic extract of *Tephrosia bracteolata* leaves, the study demonstrated that the

plant possess potent anti-pyretic activity. Egharevba *et al*., 2020 reported the antidiabetic, antioxidant and antimicrobial activities of extracts of *Tephrosia bracteolata* leaves. Other biological activities reported on other genus of the plant include anti-cancer (Hassan *et al*., 2017) and anti-plasmodial activities (Nondo *et al*., 2014). Tephrosia genus have been reported to possess several phytoconstituents that can be related to various biological activities including Isopongaflavone from *Tephrosia bracteolata* (Khalid *et al*., 1981), obovatin methyl ether from *Tephrosia aequilata* (Atilaw *et al*., 2017), kaempferol 3-O-β-D-glucopyranoside from *Tephrosia calophylla* (Ganapaty *et al*., 2009).

Pain is an ill-defined, unpleasant sensation, usually evoked by an external and internal noxious stimulus linked to tissue damage (Chatterjee *et al.,* 2015). It is physiologically associated with receptors, confirmed by electrophysiology methods in which the intensity is dependent on internal or external factors and ends up in the brain (Yam *et al.,* 2018). Additionally, pain may also be generated from peripheral and central nervous system (Grichnik *et al.,* 1991). Inflammation is a complex biological response of the body tissues to harmful stimuli like pathogens, damaged cell or irritants (Ferrero-Miliani *et al.,* 2007). The classical known symptoms of inflammation include redness, pain, swelling and heat (Medzhitov., 2008). Pain and inflammation can be classified as acute and chronic.

Analgesics are drugs that selectively relieve pain by acting on the CNS or peripheral pain mechanisms, without significantly altering consciousness (Tripathi., 2003). These drugs have serious limitation due to their side effects such as gastrointestinal irritation/ulceration, safety, tolerance, dependency and respiratory depression (Howland and Mycek., 2006). Due to these drawbacks, it is necessary to conceptualize search for newer potent analgesic and anti-inflammatory agents with better efficacy, lesser side effects, easily available and accessible.

This study aimed to establish the analgesic and anti-inflammatory effect of the aerial part of *Tephrosia bracteolata*

METHODOLOGY

Materials

Drugs and reagents

Analytical grade chemicals and reagents were used including; n-hexane, chloroform, ethyl acetate, methanol, acetic acid, sulphuric acid (Sigma-Aldrich, USA), distilled water and tween 80, Molisch's reagent, Dragendorff's reagent, Mayer's reagent and Shinoda's reagent, carrageenan (Sigma Aldrich), Acetic acid (Searle Essex, England), ibuprofen (Lek, Slovenia), pentazocine (Martinadale, Essex) and Piroxicam (Pfizer laboratories).

Experimental animals

Swiss albino mice (20-38 g) and wister rats of either sex (100-162 g) were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. They were maintained under standard conditions in propylene cages, fed with laboratory diet and water ad libitum.

Methods

Plant collection and identification

The aerial parts of the plant (T. *bracteolata*) was collected from bushy village of Samaru, Sabon gari Local Government, Kaduna State, Nigeria in October, 2018. The plant was authenticated at the herbarium unit, department of botany, Ahmadu Bello University, Zaria by comparing with voucher specimen number (0385). The aerial part was shade dried, pounded to powder and stored at room temperature for use.

Extraction and Partitioning

The pulverized plant material (2 kg) was extracted exhaustively with methanol using maceration method for four days with occasional shaking. The extract was concentrated *in-vacuo* using rotary evaporator at 40 ^{0}C which afforded 190 g greenish-brown crude methanol extract (CME). Thereafter 150 g of CME was partitioned with hexane, chloroform and ethyl acetate in order of polarity to obtain the residual fractions. The chloroform fraction was utilized for further study

Phytochemical screening

Preliminary phytochemical screening of the CF extract was investigated for the presence of alkaloids, steroids, triterpenes, flavonoids, tannins, glycosides and flavonoids using standard methods (Trease and Evans., 1996).

Oral acute toxicity profile

The median lethal dose (LD_{50}) of the CF extract was conducted according to OECD method (2000) in mice and rats. In the first phase, 3 animals were administered the extract with dose 5000 mg/kg body weight orally. The animals were observed for mortality within a period of 2 weeks. Thereafter. In

the second phase, a limit test at one dose level of 2000 mg/kg body weight of the extract is carried out with 3 animals and observed for signs and symptoms of toxicity and mortality as stated above. LD_{50} was calculated using the formula.

$LD_{50} = 20\% \, of \, maximum \, tolerated \, dose$

Antinociceptive Studies

Acetic acid induced-writhing test in mice

It was conducted according to a method described by Koster *et al* (1959). Thirty (30) albino mice of either sex were randomly divided into five (5) groups of 6 mice each. Group 1 was administered distilled water 10 ml/kg. Groups 2, 3 and 4 received 100, 200 and 400 mg/kg of the CF extract orally respectively, while group 5 received piroxicam 10 mg/kg body weight orally. An hour later, mice in all groups were administered 10 ml/kg of acetic acid (0.6 % v/v) *i.p*. Five minutes latency period was allowed and the mice were observed for abdominal writhes. The number of abdominal writhes was counted for a period of 10 minutes. Percentage inhibition of writhes was determined using equation 1

$$\% \text{ inhibition} = \frac{\text{Mean number of writhes (control)} - \text{Mean number of writhes (test group)}}{\text{Mean number of writhes (control)}} \times 100 \quad \ldots\ldots\ldots\ldots \text{ equation 1}$$

Thermal Induced Pain in Mice

The test was conducted according to the method described by Eddy & Leimbach (1953). Thirty (30) mice were divided into 5 groups of 6 mice each. The first group received distilled water as the negative control. Groups 2, 3 and 4 were administered with 100, 200 and 400 mg/kg body weight of the CF orally respectively, while group 5 received 10 mg/kg pentazocine i.p. An hour later, each mouse was placed on hot plate (Gallenkamp thermostat) kept at 45±2 ˚C. Reaction time was monitored with stop watch. Index of pain response latency was calculated as the time mouse licked its paw or jump up from the hot plate at time intervals of 30, 60 and 90 minutes after administration of drug. Baseline reaction time for each animal was recorded at zero time reading and a cut off period of 15s was observed to avoid damage to the paw.

Anti-inflammatory studies

Carragenan-induced paw oedema

Anti-inflammatory study was carried out according to the method described by Winter *et al.* (1962). Twenty-five (25) rats were divided into 5 groups of 5 rats each. Group 1 received 10 ml/kg of distilled water orally. Groups 2, 3 and 4 were administered 100, 200 and 400 mg/kg of CF orally respectively and group 5 received 10 mg/kg of ibuprofen orally. Thirty minutes later, each rat was injected with 0.01 mL of 1 % v/v solution of carrageenan in the sub plantar region of the left hind paw. The paw diameter (cm) was taken at time zero prior to treatment and subsequently measured at time 1, 2, 3, 4, 5 hours after injection of carrageenan using vernier caliper.

Statistical Analysis

The data obtained for the acetic acid-induced writhing test in mice was subjected to one way analysis of variance (ANOVA) followed by Dunnett's Post Hoc test for multiple comparisons. Also the data obtained for the thermally induce hyperalgesia and carragenaan-induced paw edema were analyzed using repeated measures ANOVA followed by Bonferoni post hoc test for multiple comparison. Results were considered significant at $P \leq 0.05$ and data was expressed as Mean±SEM.

RESULTS

Preliminary phytochemical screening:

Phytochemical constituents found present in CF were alkaloids, steroids, triterpenes and flavonoids

Acute toxicity study (LD_{50})

The oral median lethal dose (LD_{50}) value for CF was estimated to be greater than 2000 mg/kg body weight.

Acetic Acid Induced Writhing Test

The CF of the methanol aerial extract of T. *bracteolata* significantly ($P < 0.05$) attenuated the acetic acid-induced abdominal writhes in mice dose dependently. The fraction CF at dose of 400mg/kg gave highest inhibition of abdominal constriction. The standard drug piroxicam at dose of 10 mg/kg significantly ($P<0.05$) inhibited the acetic acid-induced writhes (Table I).

Table I: Effect of CF on Acetic Acid Induced Writhing Test in Mice

Treatment	Dose (mg/kg)	Mean no. of writhes ± SEM	% inhibition
DW	(10 ml/kg)	26.17 ± 5.55	0
CF	100	13.67 ± 3.24*	47.76
CF	200	13.50 ± 1.23*	48.41
CF	400	7.17 ± 1.28*	72.6
Piroxicam	10	4.33 ± 1.33*	83.4

Values presented as Mean ± SEM. Data analyzed using one-way ANOVA followed by Dunett's PostHoc test. *Significant at $p<0.05$when compared with the DW group. n = 6. CF = Chloroform fraction of the *T.bracteolata,* DW= Distilled water

Thermal-Induced Pain Test in Mice

CF significantly ($p<0.05$ and dose dependently increased the mean reaction time at dose of 400 mg/kg, at time 60- and 90-minute post treatment (Table II).

Table II: Effect of CF on Thermal-Induced Pain Test in Mice (Hot Plate)

Treatment	**Dose (mg/kg)**	**Mean reaction Time (Seconds) at various time**			
		0mins	**30mins**	**60mins**	**90mins**
DW	10 ml/kg	3.91±0.53	2.33±0.56	1.92±0.37	1.00±0.00
CF	100	3.16±0.32	7.08±1.07$^{*\#}$	6.68±1.05	6.50±1.14
CF	200	4.53±0.59	6.45±0.68	7.02±0.83	7.61±0.79*
CF	400	5.96±0.52	7.59±1.12	9.35±1.01$^{*\#}$	12.36±3.67$^{*\#}$
Penta	10	3.77±0.43	7.91±0.79*	8.47±0.47*	6.87±0.99

Values presented as Mean ± SEM. Data analysed using Repeated Measures ANOVA followed by Bonferoni's Post Hoc test. $^{\#}$Statistically significant difference ($p<0.05$) in reaction time when compared to pentazocine, *Statistically significant difference ($p<0.05$) in reaction time when compared to Distilled water n = 6. Penta = Pentazocine, CF= Chloroform fraction of the *T. bracteolata,* DW=Distilled water

Carrageenan-Induced Paw Edema

The fraction at doses of 200 and 400 mg/kg significantly ($p< 0.05$) decreased the paw edema size in rats at time 3, 4- and 5-hours post carrageenan induction when compared with the distilled treated group (Table III).

Table 3: Effect of Chloroform fraction of *T.bracteolata* on Carragenan-Induced Paw Edema Test in Rat

Treatment	Dose (mg/kg)	Mean Paw Diameter in cm (% Inhibition)				
		1 h	2 h	3 h	4 h	5h
DW	ml/kg	3.44±0.10	3.56±0.06	3.61±0.13	3.38±0.12	2.82±20.05
CF	100	3.22±0.12 (6.39)	3.04±0.17 (14.61)	2.61±0.13 (27.70)	2.50±0.13* (26.04)	2.40±0.06* (14.89)
CF	200	2.62±0.2 (19.14)	2.60±0.19* (26.9)	2.55±0.14* (29.36)	2.51±0.1 (25.74)	2.32±0.08* (17.73)
CF	400	1.74±0. 10* (46.29)	1.75±0.11 (50.84)	1.73±0.03* (52.08)	1.61±0.04* (52.37)	1.34±0.03* (52.48)
Ibu	10	1.88±0.21* (45.35)	1.86±0.06* (47.75)	1.74±0.08* (51.80)	1.54±0.07* (54.44)	1.42±0.04* (49.65)

Values presented as Mean ± SEM. Data were analysed using Repeated Measures ANOVA followed by Bonferoni post hoc test. * $p<0.05$ significant difference compared to DW group, n = 5. Ibu = Ibuprofen, CF= Chloroform fraction of the *T.bracteolata*, DW= Distilled water.

DISCUSSION

Acetic acid-induced writhing test is a very sensitive model used for evaluating the peripheral antinociceptive activity of extract at a very low dose (Shumaia *et al*., 2014). The CF in graded doses administered significantly ($p\leq0.05$) produced decrease in the number of writhes induced by acetic acid dose dependently with the highest percentage inhibition brought by the highest dose respectively. The intraperitoneal administration of acetic acid resulted in increased level of prostanoids (PGE_2 and $PGF_{2\alpha}$) and lipoxygenases (Derardt *et al*., 1980) in the peritoneal fluid thereby stimulating the nerve endings (pain) through; the release of pain mediators such as arachidonic acid via cyclooxygenase, the synthesis of chemo-sensitive niciceptors by endogenous substances such as bradykinins, prostaglandins (PGs), serotonin and histamine (Davies *et al*., 1984). The model is thought to involve in part local peritoneal receptors (Bentley *et al.*, 1983). The CF extract may have interfered with these peritoneal receptors to bring about analgesia (Musa *et al.*, 2009). The analgesic activities exhibited by the CF extract may occur through the process of inhibiting the production of those algogenic substances or its action on visceral receptors sensitive to acetic acid. The extract produced strong analgesic effect on the acetic acid-induced writhing in the same order of magnitude as that observed by piroxicam administration.

Thermally induced hyperalgesia is a specific model for testing centrally mediated antinociception and the observed activity is selective for compounds acting through opioid receptor (Kaushik *et al*., 2012). The increase in the mean latency to pain by CF in the thermally-induced pain in mice suggests that the extract possesses centrally acting analgesic activity (Kaushik*et al*., 2012). At dose of 400 mg/kg, CF at 60 and 90 minutes exhibited greater activity when compared to the standard agent pentazocine at 10 mg/kg. This suggests that the extract may act via centrally mediated mechanism (Kaushik *et al*., 2012). The extract (100 mg/kg) at 30 minutes produced a significant effect ($p<0.05$) on the pain threshold on the test group when compared to the positive control, suggesting that the activity of the extract is dose dependent.

The process of inflammation is multiphasic including; the increase in vascular permeability, leucocytes infiltration and the formation of granuloma. Carrageenan is a phlogistic agent that induces edema in the paw of experimental animals used as a model for testing anti-inflammatory activity (Chatterjee *et al.,* 2015). The early phase (1 hour) of carrageenan induction in the sub-plantar region of the paw is mediated by the release of histamine, serotonin and kinins. The late phase (2-3 hours) is mediated by the release of prostaglandins and lysosome enzymes (Ahmed., 2011).

CF extract contains flavonoids, several derivatives of flavonoids are known to exhibit anti-inflammatory and analgesic activities by inhibiting the enzyme prostaglandin synthetase, more specifically the endoperoxidase reducing the release of arachidonic acid (Derardt *et al*., 1980).

Therefore, the anti-inflammatory activity exhibited by CF extract may be due to the inhibition of COX1 and COX2 that converts arachidonic acid to PGG2 and PGH2 leading to the production of thromboxane A2 (Burke *et al*., 2006). This process is similar to that of NSAIDs (Roberts and Morrow, 2001) thus validating the ethnomedical use of the plant in

achieving analgesia and treating inflammatory conditions.

The result of the oral acute toxicity study of the CF extract shows that after the administration of 2000 mg/kg of the extract, no lethality and mortality were observed. The LD_{50} was therefore determined to be 2000 mg/kg which is relatively non-toxic. The phytochemical screening revealed the presence of alkaloids, triterpenes and flavonoids which may be responsible for the observed activities (Kaushik *et al*., 2012).

The analgesic effect of the extract may be due to the presence of triterpenes, flavonoids, alkaloids or saponins (Kaushik *et al*., 2012).

CONCLUSION

These findings in this experiment substantiated that the CF of the methanol aerial extract of *Tephrosia bracteolata* contain bioactive phytochemicals with analgesic and anti-inflammatory activities, and further support the ethnomedical claim of the use of the plant in the management of pain and inflammation.

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