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Toxicity and antipyretic studies of the crude extract of *Tephrosia bracteolata* leaves

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Abstract

The acute toxicity of the crude methanolic extract of *Tephrosia bracteolata* was determined and the antipyretic activity was evaluated in rats induced with fever by subcutaneous injection of 12% yeast suspension, at the dose of 1mg / kg body weight. The rectal temperatures of the rats were taken using clinical thermometer inserted into the rectum at hourly intervals for six hours. The extract was found to be safe with L_{D50}>5000 unit and the dose of 600 mg/ kg body weight administered intraperitoneally, exhibited the most potent antipyretic activity within the first 2 hours post administration. The antipyretic activity of the crude extract compared very well with standard antipyretic drug, aspirin, injected intraperitoneally at 50 mg/kg body weight. The results suggest that the crude methanolic extract is safe and contains active compounds with marked antipyretic activity.

Key Words: - *Tephrosia bracteolata*, methanolic extract, acute toxicity, antipyretic, rats, mice.

Introduction

Tephrosia bracteolata, (Gill and Perry) is a legume belonging to the family pappilanacae and it is commonly found in uncultivated areas of Northern and Southern Nigeria [1, 2]. The plant is traditionally employed for various purposes by local people, including the treatment of whitlow, open wounds, ear and tooth aches. The leaves are also given as feed to horses, sheep [1] and goats [3].

The leaves of this plant have been previously reported to be toxic by Jubril [4], but Onaolapo *et al.* [5,6] reported that they are non toxic (L_{D50} > 5,000 mg/ kg), and that they posses analgesic and anti inflammatory activities. It was also discovered that Jubril [4] pulverized the leaves of the plant together with the pods containing the seeds; hence his assertions cannot be for the leaves alone. The aim of the present study was to evaluate the antipyretic activity of the leaves of *Tephrosia bracteolata,* and thus confirm or otherwise the claims of the traditional medical practioners on the plants.

Material and methods

Fresh whole plants of *Tephrosia bracteolata* were collected from the open lands around the Institute for Agriculture Research (I.A.R), Zaria and residential Area F of the Ahmadu Bello University (A.B.U), Zaria. The plant was authenticated by Mallam M.D Musa, of the herbarium, Department of Biological Sciences A.B.U Zaria. A Voucher specimen of the plant (Herbarium number: 776) has been deposited at the Department of Veterinary Physiology and Pharmacology, A.B.U., Zaria.

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The leaves were separated and allowed to dry openly in the sun for about one week. The dried leaves were pulverized with 1.0mm filter at the National Animal Production Research Institute A.B.U., Zaria, using Christy Laboratory grinding machine.(Christy and Norris Limited Process Engineers, Chemsford, England).

Extraction

About 35.5g of pulverized leaves of the plant was extracted continuously for 12 hours using 95% methanol in a soxhlet extractor. The methanolic extract was concentrated using Buchi rotatory evaporator, and kept in a desiccator to dry completely. It gave a percentage yield of 21.3.

Experimental animals

Wistar rats (140 280)g of both sexes and about 10 12 weeks old served as subjects of the experiment. They were purchased from the Department of Veterinary Physiology and Pharmacology, A.B.U., Zaria. The rats were fed on standard feed, purchased from Pfizer feed Lagos, Nigeria. The animals were kept in groups of five in plastic cages, and given free access to feed and tap water *ad libitum* during the preconditioning period of two weeks.

Toxicity studies

The Method of Lorke (7) was employed in which three different doses (10,100 and 1000mg/kg) of the crude extract were administered to adult mice and rats respectively, by using a curved needle to which a cather has been fixed. Three animals were chosen for each dose level. A fourth group containing 3rats and 3 mice respectively was administered 0.2ml of distilled water, to serve as the control. The animals were closely monitored every 30 minutes, for the first 3 hours after administration of the crude extract and hourly for the next 6 hours, observing to see any adverse effects and the time of occurrence. The animals were then observed for another 7 hours.

The second stage of 1,600, 2,900 and 5,000 mg/ kg dose levels of the crude extract were administered to the three groups of fresh adult rats and mice respectively, by using a curved needle to which a cathereter has been fixed. In each group only one animal was required for the test. The fourth group consist of a rat and a mouse and was administered 0.2ml distilled water to serve as control. The animals were observed every 30 minutes for first 3 hours, then hourly for another 6 hours and further observations for 72hours. All the animals were sacrificed and post mortem examination conducted at the termination of the studies. For all animals used, food and water were withdrawn 12 hours before the administration of crude extract of *Tephrosia bracteolata* leaves.

Antipyretic activity

Antipyretic study was carried out using the methods of Ramadan *et al*[8] and Awe *et al.*,[9]. Thirty five Wistar rats divided into seven groups, of 5 per group were used. Rats in group 1 were not induced with pyrexia. Pyrexia was induced in rats belonging to groups 2-7 by subcutaneous injection of 12% yeast suspension at the dose of 1mg/kg body weight. Post induction of pyrexia, feed and water were withdrawn from all the animals for 15 hours. Thereafter, the 0 hour recording of rectal temperatures (RT) of the rats were taken using a clinical thermometer, calibrated in degree Celsius, (^{0}C). The RT recording was repeated at hourly intervals for six hours. The experimental groups were treated as follows: Group 1

served as control and pyrexia was not induced in the rats. Group 2 was given 0.2ml distilled water intraperitoneally, and they served as control II. Group 3 received 50mg /kg body weight of aspirin intraperitoneally. Groups 4, 5, 6, and 7 received intraperitoneally 200, 400, 600, and 800 mg /kg body weight of the crude extract of *Tephrosia bracteolata* leaves respectively, 15 hours post pyrexia induction.

Statistical analysis

All the data obtained were expressed as means + standard error of the means (S.E.M), and values of $P<0.001$, $P<0.01$ and $P<0.05$ were considered significant, using and students t test, correlation analysis and analysis of variance (ANOVA).

Results

Toxicity Study

From Table 1, for all the animals, no visible adverse reactions were observed up to 72 hours post administration. In all cases, no death was recorded, even at a dose of 5,000 mg/ kg body weight. When the animals, which were apparently healthy, were sacrificed and posted, no gross and histological lesions were seen in the organs. The restlessness observed in the rats when higher doses of 1,600 5,000 mg/kg body weight were given may be attributed to the high volume of aqueous extract administered to the animals (>0.8ml) since they all recovered within 5 minutes and resumed eating. The observation that a dose greater than 5,000mg / kg body weight did not produce adverse effects implies that *Tephrosia bracteolata* is not toxic and it is safe for human and animal consumption. The high safety level is favourable for the widespread use of the plant among the local people as live stock feed and as pain reliever.

Table 1: Evaluation of crude extract of *Tephrosia bracteolata* leaves toxicity in rats and mice administered intraperitoneally.

Dose mg/kg	No of Deaths	No of Animals Per Rest	Survival	Mortality Ratio
10	0	3	3	0/3
100	0	3	3	0/3
1,000	0	3	3	0/3
1,600	0	1	1	0/1
2,900	0	1	1	0/1
5,000	0	1	1	0/1
10,000	0	1	1	0/1
Control	0	3	3	0/3

Antipyretic activity

From table 2, the dose of 600mg/kg body weight of the crude extract *Tephrosia bracteolata* leaves was the most potent. The RT of rats administered with this dose, 6h post administrations, was the lowest with the value of $37.5 + 0.1^{0}C$. Close to this value was the RT of $37.7 + 0.1^{0}C$ obtained from rats given 800mg/kg of the crude extract. The RT of rats treated with yeast only, that is, pyrexia induced rats, is significantly higher ($P<0.01$) than that obtained from those administered 400mg/kg of *Tephrosia bracteolata* crude extract ($P<0.01$), and especially rats given 600mg /kg ($P<0.001$). Although the RT was low in rats treated with 200mg/kg of the crude extract, the value was not significantly different from that obtained in rats treated with yeast only. There was a negative correlation between the time elapsing post injection of the crude extract and the temperature of the rats treated with 600 mg/kg ($r = 0.738$, $P<0.05$) and those injected with 800mg/kg ($r = -0.924$, $P<0.001$). Although the correlation coefficient between the time elapsing post injection of the extract

and the RT of the rats administered with 400mg/kg *Tephrosia bracteolata* leaves crude extract was positive (r = 0.331, P>0.05), the relationship was not significant. The lowest correlation coefficient of 0.924 (Table 3) was found between the time elapsing post administration of the extract and the RT of the rats injected with 800mg/kg of the extract. The correlation between the RT induced rats and that of rats administered 50mg/kg aspirin was negative and insignificant. Also, the relationship between the RT of the controlled II (rats not induced with pyrexia) and pyrexia induced but treated with 200 mg/kg body weight of the crude extract of *Tephrosia bracteolata* leaves was 0.786 (P< 0.05). In pyrexia induced rats, treated with 400mg/kg and those treated with 600mg/kg, the relationship was negative and insignificant (r= -0.284 and - 0.182, respectively). It appears, therefore that the optimum dose for this crude extract in rats was 600mg/kg.

The results obtained for the different doses of the crude extract of *Tephrosia bracteolata* leaves at 200, 400, 600 and 800mg/kg body weight compared very well in efficacy with that of the standard drug, aspirin. From Table 1, the crude extract of *Tephrosia bracteolata* leaves was most potent at the dose of 600mg/kg. In general, the antipyretic activity of the crude extract was highest within the first 2 hours after the administration of the crude extract of the leaves.

Table 2: Rectal Temperature Responses with Time in Pyrexia-induced Rats treated with and without Tephrosia bracteolata Crude Extract oc, (Mean ± S.E.M., n = 5)

Time (Hours)	Control I (Pyrexia not induced)	Control II (Rats induced with pyrexia)	50 mg/ kg ASA	*Tephrosia bracteolata* Crude Extract, mg/ kg			
				200	400	600	800
0	38.10±0.00	38.40±0.03	8.26±0.09	38.34±0.08	38.34±0.04	38.20±0.15	38.10±0.28
1	38.10± 0.00	38.54± 0.09	38.38± 0.34	37.58± 0.16	37.44± 0.35	37.56± 0.17	37.76± 0.13
2	38.10± 0.00	38.74± 0.15	38.50± 0.19	38.10± 0.25	37.64± 0.33	37.30± 0.12	37.66± 0.21
3	38.10±0.00	38.72±0.15	38.76±0.19	38.54±0.24	37.80±0.29	37.40±0.13	37.68±0.27
4	38.10± 0.00	38.88± 0.19	38.40± 0.21	38.38± 0.18	38.24± 0.15	37.38± 0.05	37.66± 0.25
5	38.10± 0.00	38.48± 0.19	38.50± 0.10	38.42± 0.15	38.14± 0.22	37.32± 0.06	37.48± 0.13
6	38.10±0.00	38.08±0.37	38.52±0.14	34.44±0.20	34.16±0.22	37.30±0.10	37.32±0.07
Mean ± S.E.M	38.10± 0.00	38.56± 0.1	37.68± 0.55	37.68± 0.60	37.97± 0.13	37.49± 0.12	37.67± 0.10

ASA = Acetylsalicylic acid (Aspirin), n = number of rats in each group.

Table 3: **Statistical Comparison of Temperature Values Obtained for the Crude Extract of Tephrosia bracteolata Treated Rats, and those with the Standard Drug (aspirin) and the Untreated Control.**

Groups	t - Value	P- Value	Remarks
1 Vs 5	0.640	>0.05	Not Significant
2 Vs 5	7.432	<0.001	Very significant
6 Vs 3	3.826	<0.01	Very significant
5 Vs 1	6.660	<0.001	Very significant
5 Vs 4	3.850	<0.01	Very significant
4 Vs 6	2.660	<0.05	Significant

Groups
1 - Untreated Control
2 - Aspirin Treated
3 - 200mg/kg Extract Treated
4 - 400mg/kg Extract Treated
5 - 600mg/kg Extract Treated
6 - 800mg/kg Extract Treated

Extract - *Tephrosia bracteolata* Crude Extract
t Value - Calculated students t test value
p- Value - Level of statistical significance

Discussion

The result of the present study show that the leaves of *Tephrosia bracteolata* is safe and provides scientific basis for the administration of administration of *Tephrosia bracteolata* leaves by traditional medical practitioners in Nigeria and countries in West Africa sub region in the treatment of purulent disease conditions, often associated with increase in local or general body temperature. The fact that no mortality occurred in all experimental animals administered 500mg/kg body weight during acute toxicity studies indicated that the crude methanolic extract of *Tephrosia bracteolata* leaves was not toxic in rats and mice. This shows that the crude extract is safe for human consumption [7]. Indeed according to the results of nutrition investigation conducted by Adeloye [3] , the plant *Tephrosia bracteolata* leaves is good as sole feed for goats. Also this study has demonstrated that the crude extract of *Tephrosia bracteolata* leaves has significant antipyretic activity, which is comparable with that of aspirin (50mg/kg) activity ($P<0.001$). Therefore, the methanolic crude extract of *Tephrosia bracteolata* leaves may contain a potent antipyretic agent. The present findings support the result of the previous works of Onaolapo *et al.*, [5,6] who demonstrated that the crude extract also possesses analgesic and anti-inflammatory activity. This result suggests that the crude methanolic extract of *Tephrosia bracteolata* leaves contains active compounds with marked antipyretic activity. Further studies which will involve isolation and characterization of the active compounds contained in the crude extract of *Tephrosia bracteolata* leaves coupled with our findings would lead to design of potent drug for therapy and prophylaxis of disease conditions associated with increase in general or local body temperature

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