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Acute Toxicity and Analgesic Studies of Crude Aqueous Extract of *Nicotiana tabaccum* Leaves in Wistar Albino Rats and Mice

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Abstract:
The dried leaves of Nicotiana tabaccum leave was extracted in water by maceration method. The aqueous extract was then subjected to acute toxicity study by lorke's method in Wistar albino rats through intraperitoneal route; and analgesic activity study by hot plate method in mice. The acute toxicity study showed the extract has low toxicity, with LD_{50} value of 471.2 mg/kg body weight. The result of the analgesic activity study showed significant ($P < 0.01$) increase in reaction time (in seconds) to the thermally-induced paddling, from 6.80 ± 1.10 to 11.20 ± 1.64 with corresponding percentage increase in pain threshold of 47.8 to 143.4 % for the extract doses used (50 to 100 mg/kg body weight) compared with the control. The overall analgesic findings showed the crude aqueous extract has greater analgesic activity (143.4 % increase in pain threshold at 100 mg/kg) than the standard drug, pentazocine (126.0 % increase in pain threshold at 20 mg/kg). It was concluded that the prejudices generated by the ill-effects of tobacco smoking should be set aside, so that tobacco leaves be systematically examined for substances of therapeutic value, as all useful agents are beneficial only when used in positive ways but if abused will be detrimental.

Key words: *Nicotiana tabaccum, extract, toxicity, analgesic, pain.*

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1. INTRODUCTION

Plant based natural compounds have long history of providing preventative and curative benefits against numerous diseases (Zhang and Reddy, 2018). Plants in their diversity are a gift of nature to

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man and his livestock and even crops, as plant and plant products have the history of application on human, animal and crops for the treatment of different diseases and control of pests (Ouadja *et al*., 2018). Today, natural products isolated from plants have been postulated to remain an essential part of the search for novel medicines against human and animal diseases (Semenya *et al*., 2018).

Tobacco (*Nicotiana tabaccum*) plant, though has been reported to be probably responsible for more deaths than any other herb as a result of irrational use (Binorkar and Jani, 2012), never the less is also known to possess several valuable medicinal properties. Indeed, in 1500s onwards tobacco has acquired a reputation as a panacea, to the extent of being called the 'holy herb' and 'God's remedy' (Dickson, 1954). Review of publications shows tobacco was long used as orthodox medicine by the members of the medical profession (Charlton, 2004). Documented reports show tobacco leaves have been used with success in the treatment of different ailments which include ulcerated abscesses, fistulas, sores, inveterate polyps, catarrh, colds, intermittent fevers, constipation, haemorrhoidal bleeding, strangulated hernia, malaria, dislodging obstructive material from oesophagus, ulcers, wounds, migraine and toothache (Stewart, 1967; Charlton, 2004). In fact, according to Decaux during the sixteenth century there were few ailments for which tobacco was not prescribed (Decaux, 1961).

To date, the local people of Northern Nigeria still greatly value the leaves of *N. tabaccum* in the treatments of fevers, general body pains and particularly toothache, by chewing (and holding the substance in the mouth) without swallowing of the fermented leaves of this plant. The aim of this study was to evaluate the toxic and analgesic properties of the crude aqueous extract of *N. tabaccum* leaves in Wistar albino rats and mice, respectively.

2. MATERIAL AND METHOD

Extraction of Plant Material

The leaves were first air-dried under the shade at ambient temperature and pulverized to powder using a clean grinding machine. Maceration method was used for the extraction. One hundred grams (100 g) of the powdered sample was blended with 2.5 litres of distilled water in a 5 litre round bottom flask for 48 hours with agitation at room temperature. The mixture was decanted and the solution filtered using Whatman filter paper No. 1. Some fresh distilled water was added to the residue and allowed to stand for 24 hours, decanted and filtered. The solutions were combined and transferred into an open tray, and dried in an oven at 40^0C, for 24hours. The dry extract was weighed and kept for further analysis.

Experimental Animals

Twenty five (25) mice of both sexes weighing 20-25 g were used for the analgesic study, whereas thirteen (13) Wistar albino rats of both sexes weighing between 105 -158 g were used for the acute toxicity study. Animals were kept for acclimatization period of two weeks, and handled according to global best practices.

Acute toxicity study

The acute toxicity (LD_{50}) was done through intraperitoneal (i.p) route according to Lorke's method

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(Lorke, 1983). In phase one, 9 rats were randomly divided into 3 groups of 3 rats per group each and administered the extract at the dose rate of 10, 100 and 1000 mg/kg body weight i.p, respectively. The rats were observed for 24 hrs for signs of adverse effects such as paw-licking, salivation, rubbing of nose on the floor and wall of cage, change in body weight and death. In the second phase 4 rats were divided into 4 groups of 1 rat each and administered (i.p) the extract at the doses of 100, 225, 370 and 600 mg/kg body weight, respectively and observed again for 24 hrs for signs of toxicity. The number of deaths in each group within 24 hrs was recorded and the final LD_{50} value was calculated using the formula:

$LD_{50} = \sqrt{a \times b}$

a = lowest dose that kills an animal

b = highest dose that did not kill any animal

Procedure for Testing Analgesic Activity by Hot Plate Method (Paddling Method)

The hot plate method was based on individually placing the animal on a hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds (Eddy and Leimbach, 1953). The animals were divided into five groups of 5 animals each. Group I served as negative control and received 1 ml distilled water orally. Group II, III and IV were treated orally with the aqueous extract at 50, 100 and 200 mg/kg body weight, respectively. Group V served as positive control and were injected pentazocine (30 mg/kg), intraperitonially.

Data Analyses

The results were analysed using GraphPad Instat Version 3.05, 2000 and presented as means ± standard deviation (SD). Differences between means were assessed using Analysis of variance (ANOVA) and post-test using Dunnett comparison test (Mead and Curnow, 1982). $P < 0.01$ or $P < 0.05$ was considered significant.

3. RESULTS

Table 1. LD_{50} value of crude aqueous extract of *N. tabacum* leaves in Wistar albino rats by intraperitoneal route.

Phase	No. of rats	Dose (mg/kg)	Mortality
1	3	10	0/3
1	3	100	0/3
1	3	1000	3/3
2	1	140	0/1
2	1	225	0/1
2	1	370	0/1
2	1	600	1/1

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In phase one of the study, there was no mortality in all the groups. The mortality occurred in the second phase of the study at the extract dose of 600 mg/kg (Table 1).

The LD_{50} was thus calculated as $LD_{50} = \sqrt{a \times b}$

Where a = 370 mg (highest dose that did not kill the animal)

b = 600 mg (least dose that kills the animal)

$= \sqrt{370 \times 600}$

$= \sqrt{222{,}000}$

= 471.2 mg/kg.

The LD_{50} of the crude aqueous extract of was therefore evaluated to be 471.2 mg/kg intraperitoneally.

Table 2. Effect of the Crude Aqueous Extract of *N. tabacum* Leaves on Thermally-induced Paddling in Mice.

Group	Rx	Dose (mg/kg)	Reaction time in seconds at 30 min. (mean ± SD)	Increase in pain threshold (%)
Control	NS	.....	3.80 ± 0.45	.....
Test – 1	ET	50	6.80 ±1.10**	47.8
Test – 2	ET	80	9.00 ±0.71**	95.6
Test – 3	ET	100	11.20±1.64**	143.4
STD	P	20	10.40±1.14**	126.0

Values are mean number of seconds ± SD (n=5 per group).

** P < 0.01 significantly different from control group.

Keys: STD = Standard, Rx = Treatments, NS = Normal saline, ET = Extract, P = Pentazocine.

The effect of crude aqueous extract of *N. tabacum* leaves on thermally-induced paddling in mice is presented in Table 1. The result showed significant (P < 0.01) increase in reaction time, in seconds of 6.80 ± 1.10 to 11.20 ± 1.64 for the corresponding extract doses of 50 to 100 mg/kg body weight, compared with the control (3.80 ± 0.45). The percentage increase in pain threshold for the same extract doses (50 to 100 mg/kg body weight) was 47.8 to 143.4 %, compared with the control. The standard drug, pentazocine showed increase in reaction time of 10.40 ± 1.14 seconds with percentage increase in pain threshold of 126.0 %.

4. Discussion

The toxicity study of crude aqueous extract of *N. tobacum* leaves in Wistar albino rats showed the parenteral (i.p) LD_{50} was 471.2 mg/kg. According to Clarke and Clarke, when classifying toxicity of substances according to LD_{50} values, substances with oral LD_{50} values of 1000 mg/kg body weight and intraperitoneal LD_{50} values of 500 mg/kg body weight can be said to be of low toxicity and therefore safe (Clarke and Clarke, 1977). This intraperitoneal LD_{50} (471.2 mg/kg) value suggests the crude extract was of low toxicity parenterally.

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By employing one way ANOVA, all data were found to be statistically significant ($p<0.01$). Analgesic effect against thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomena. In this study, the crude aqueous extract of *N. tobacum* showed a dose dependent activity, percentage increase in pain threshold increasing from 47.8% to 143.4% for the extract doses of 50, 80 and 100mg/kg, respectively. However, the extent of activity shown by the crude aqueous extract at the dose of 100 mg/kg was found to be greater than that of the standard drug, Pentazocine (126.0% increase in pain threshold at 20 mg/kg). This suggests that the crude aqueous extract of *N. tobacum* leaves possesses strong analgesic property against thermally induced stimulus in mice. The overall analgesic findings on the crude aqueous extract of *N. tobacum* leaves in this study agrees with the report of Charlton (2004) where she reported strong pain killing activity of *N. tobacum* leaves.

5. Conclusion

Tobacco leave is still utilized for the treatment of some ailments in some parts of the world traditionally but has long been removed from pharmacopoeias and from medical practice due to the nicotine content which is lethal in small doses (Royal College of Physicians, 1977). But it is worth noting that with any useful agent, excess dosage will do harm. However, if used in positive ways it will heal and protect; but if abused, will also have the power to harm. Therefore as suggested by Charlton (2004) 'the prejudices generated by the ill-effects of tobacco smoking should be set aside, let tobacco leaves be systematically examined for substances of therapeutic value'.

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