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ORAL ACUTE TOXICITY STUDIES ON ACTION BITTERS IN ALBINO RATS

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ABSTRACT

In this work, the oral acute toxicity profile of Action bitters in albino rats was studied. The studies were conducted in two phases, phase one (1) and phase two (2). A total number of twenty one (21) albino rats were used. These were randomly distributed into seven groups. Group one (1) to four were used in phase 1 while group five (5) to seven (7) were used in phase two. In phase 1 study, the first group were administered with distilled water and used as control while groups two to four were treated with 2, 4, and 6 ml/kg b. w of the Action bitters respectively. All animals were observed for the first twenty four hours for toxicity signs and mortality. From the result obtained, the doses of the action bitters were increased to 12, 16 and 20 ml/kg b. w in phase 2. At the end of 24 hours, toxicity and mortality were also recorded. All animals were observed for 72 hours at the end of which they were sacrificed. Blood samples were taken for the determination of haemoglobin (Hb) level and Packed cell volume (PCV). Results showed that at maximum dose, 20ml/kg b. w (phase 2), only shivering and paw licking were observed without death occurring. There were also slight changes in body weight of the animals. Results of the haematological analysis revealed significant reduction ($P<0.05$) in both Hb and PCV. It was thus concluded that the median lethal dose (LD_{50}) of action bitters in albino rats lies within a range above 20ml/kg b. w.

KEYWORDS: Acute toxicity, Action bitters, Haemoglobin, lethal dose.

INTRODUCTION

The discovery that compounds derived from plants could act as potential therapeutic weapons against various human, animal and even plant diseases, in addition to their food and nutritional values, has made plants invaluable and indispensable to human and animal lives (Ogbonnia *et al*., 2008). Plants with therapeutic activities are known as medicinal plants and remain the main source of the active drugs from natural sources. Plant derived medicine is popularly known as herbal or phytomedicine. Herbal medicine is renowned as the most common form of alternative medicine and is used by about 60% of the world population both in the developing countries and in the developed countries where modern medicines are predominantly used (Rickert *et al.,* 1999; Ogbonnia *et al.,* 2008). The use of herbal medicines by the traditional practitioners for treatment of diseases remains the main stay of health care system and is gaining increasing popularity especially among the rural populace in the developing countries. Its rising popularity could be attributed to its advantages of being efficacious and also a cheap source of medical care. There is also growing disillusion with modern medicine coupled with the misconception that herbal products being natural may be devoid of adverse and toxic effects associated with convectional and allopathic medicines. of many plant parts obtained from various plant species and families and may contain multiple bioactive constituents that could be difficult to characterize.

The bioactive principles in most herbal preparations are not always known and there could be possibilities of interaction with each other in solution. The quality as well as the safety criteria for herbal drugs may be based, therefore, on a clear scientific definition of the raw materials used for such preparations. Also herbal medicine may have multiple physiological activities and could be used in the treatment of a variety of disease conditions (Pieme *et al.*, 2006). They may be administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage. The danger associated with the potential toxicity of such therapy and other herbal therapies used over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs (Tedong *et al.,* 2007). There were various claims on the efficacy of action bitters in curing diseases like Cirrhosis, Kidney failure, diabetes, waist pain, typhoid fever, Menstrual pain as well as convulsions, constipation and stomach ache but there were not many work on the toxic effects of these bitters. Hence, this study determined the toxic effects of action bitters.

The aim of this research work is to determine acute toxicity of action bitters on albino rats.

MATERIALS AND METHODS

Collection of Samples: A 100ml pack of action bitters was purchased from a shop at Nekede and was taken to the laboratory for the study. The material was stored in a refrigerator at 4^0C and protected from light until time of administration when it was allowed to warm up to room temperature. Upon the attainment of room temperature (27 ± 2°C), the appropriate doses of the bitters were administered orally to the experimental animals.

3.5 Experimental Animal: A total of twenty one (21) males albino rats of average weight 100.5± 1.50g were obtained from the animal holdings of the department of Biochemistry University of port-Harcourt (Uniport) and used for the studies.

3.6 Measurement of Body Weight: The body weight of all the animals were measured and recorded before commencement of the studies (after 4 days of acclimatization in the laboratory).

3.7 Experimental Design/ Treatment of Animals: The oral acute toxicity studies were done using twenty one (21) albino rats of both sexes in two phases. The animals were randomly distributed into seven (7) groups of three (3) albino rats per group.

In phase 1 of the study, the first three (3) groups were administered with 2, 4 and 6ml /kg body weight of the action bitters.

In phase 2, the remaining two (2) groups were given the following doses 12, 16 and 20ml/kg body weight of the action bitters.

These treatments were given after overnight fasting. The number of death in each group was observed within 24 hours for a maximum of 48 hours. The LD_{50} was estimated according to the method of Lorke's (1983). The outlay of the work is as shown below.

Phase. 1.

Groups	Doses of Action Bitters (ml/kg body weight)	Number of Rats
1	Control (Distilled water)	3
2	2	3
3	4	3
4	6	3

Phase. 2.

Groups	Doses of Action Bitters (ml/kg body weight)	Number of rats
5	12	3
6	16	3
7	20	3

After seventy-two (72) hours all surviving animals were weighed again and subsequently sacrificed. The blood samples were taken to the laboratory for the measurement of Hb and PCV levels.

3.8 Measurement of Hb Concentration: Haemoglobin (Hb) estimation was done by the cyanomethaemoglobin method described by Jain (1986). 0.02ml of blood was added to 5ml of Drabkin's solution in a test tube (1:250) and mixed thoroughly. This was allowed to stand for 10 minutes. Absorbance was read at 540nM against a reagent blank (Drabkin's solution). Absorbance of known standard (Borine Bb) was also taken alongside samples as control. The known standard contain 14.6 g/dl of whole blood. The sample haemoglobin concentration was read off using a table prepared from the calibration graph.

Packed Cell Volume (PCV)

Determination of PVC was done using capillary tubes with blood as described by Dalie and Lewis (1991). The capillary tube was filled with well mixed blood, scaled at one end with plasticine and then placed in the micro-haemotocrit centrifuge and spun at 10,000 rpm for 5 minutes. The spun tube was then placed in the scale (reader) and the PVC was calculated as;

$$PVC\ (\%) = \frac{Volume\ of\ Rbc\ in\ given\ vol.\ of\ blood}{Total\ Blood\ Volume} \times 100$$

RESULTS

Table 1: Effect of Acute Toxicity Treatment on Mortality Pattern.

Groups	Doses of Action Bitters (ml/kg body weight)	Number of Rats	Number of Deaths
1	0 Control	3	0 (Phase 1)
2	2	3	0
3	4	3	0
4	6	3	0
5	12	3	0 (Phase 2)
6	16	3	0
7	20	3	0

The LD_{50} of action bitters in albino is thus estimated to be > 20 ml/kg body weight.

Table 2: Effect of Acute Toxicity Treatment on Body weight of albino Rats.

Groups	Doses of Action Bitters (ml/kg body weight)	Weight Before Treatment (g)	Weight 72 Hours After Treatment (g)
1	0 CONTROL	95.3±1.0	95.5±1.1 (Phase 1)
2	2	102.0±0.9	102.1±0.8
3	4	106.0±1.1	105.0±1.2
4	6	100.1±1.0	101.0±0.6
5	12	98.2±1.1	90.0±1.5 (Phase 2)
6	16	100.0±0.9	94.2±1.0
7	20	98.2±0.8	93.6±0.9

Values represent mean ± S. D. (n=3).

Table 3: Effect of Acute Toxicity Treatment on Selected Haematological indices of albino Rats.

Groups	Doses of Action Bitters (ml/kg)	Haemoglobin Level (Hb) (g/l)	Packed Cell Volume (PCV) Level (%)
1	0 (CONTROL)	93.3 ± 1.9^{a}	32.0 ± 0.1^{a}
2	2	90.20 ± 0.8^{a}	30.5 ± 0.4^{a}
3	4	91.71 ± 1.5^{a}	31.2 ± 0.2^{a}
4	6	89.50 ± 0.5^{a}	28.7 ± 0.9^{a}
5	12	90.30 ± 0.1^{a}	29.5 ± 1.0^{a}
6	16	76.51 ± 0.8^{b}	21.6 ± 0.2^{b}
7	20	60.30 ± 1.2^{c}	20.0 ± 1.1^{b}

Values represent mean ± S. D. (n=3).

a, b, c: values with different superscript indicate significant difference ($p<0.05$).

DISCUSSION

Herbal medicines have received greater attention as alternative to clinical therapy in recent times, leading to subsequent increase in their demand (Sushruta et al., 2006). In rural communities, the exclusive use of herbal drugs prepared and dispensed by herbalists without formal training, for the treatment of diseases is still very common requiring that experimental screening be established to ascertain the safety and efficacy of these herbal products as well to establish their active components (Ogbonnia *et al.*, 2008).

The acute toxicity study of the bitters presented some behavioural changes in the animals at the highest dose of 20ml/kg b. w. this changes include shivering, change in mood, paw licking and loss of appetite. However, up to this maximum dose studied, no mortality was recorded. This observation therefore suggest that the oral median lethal dose (LD_{50}) value of

action bitters in albino rats lies within a range above 20ml/kg b. w. action bitters also elicited some remarkable variations on the body weight of the animals.

In Phase 1 of the study, when the drug was given at a dose 2ml/kg b. w, the body weight of the animals slightly changed from 95.3±1.0 to 95.5±1.1. Up to the dose of 6ml/kg b. w, a change in body weight was also not remarkable (100.1±1.0 to 101.0±0.6).

In the second phase of acute toxicity study, the dose effect of the bitters on body weight of the animals became most profound at the highest dose of 20ml/kg b.w (the body changes from 98.2±0.8 to 93.6±0.9). This remarkable reduction in body weight of the animal in the highest dose group could be attributed to the reduction in feed intake as a result of loss of appetite. The result observed in the haematological parameters is in collaboration with that of Onwusonye *et al.*, 2014 in which he demonstrated that acute toxicity of albino mice with methanol extract of *Annona senegalencies* was able to reduce the level of Hb and PCV. In this study, the changes in the haemoglobin level were however very significant from the dose level of 16ml/kg b. w upwards. Haemoglobin (Hb) gives a direct index of the total blood volume in circulation throughout the body of an animal.

This study has shown that single oral acute doses of action bitters in albino rats were able to elicit a significant reduction in Hb level of the animals. This reduction in Hb level could be attributed to haemolysis (destruction of the red blood cell) brought about by the alcohol content of the action bitters. The effect of the acute dosing of the action bitters on the PCV level of the albino rats also occurred in the same pattern as those of the haemoglobin level. At the lowest dose 2ml/kg b. w used, the PCV level was 30.4±0.4%. when the action bitters was administered up to the maximum dose of 20ml/kg b. w, pack cell volume was significantly reduced to 20.0±1.1% ($p<0.05$).

For a long time in history, alcohol has been consumed by man for various occasions and for various reasons. Health and safety demands that before a new product is certified fit for use or for consumption, detailed study need to be done on its safety/toxicity profile. This will make for safe use of drugs, chemicals and food products. Drug related misbehaviours on increase especially among youths of these days could be traced to use of such without cognizance to the appropriate doses and conditions of use. From this study, action bitters which is commonly consumed by youths has been demonstrated to be linked with pronounced toxicity effect as already pointed out.

CONCLUSION

The median lethal dose (LD_{50}) values of action bitters have been observed to be above 20ml/kg b.w. Toxicity symptoms observed include: change in mood, loss of appetite and paw licking. Action bitters has equally been observed to elicit significant reduction in body weight, haemoglobin and packed cell volume levels of albino rats. However, doses below 20ml/kg b. w. of action bitters could thus be considered as safe substance and should be among the GRASS while doses above 20ml/kg b. w. of the action bitters is toxic and as such should not be among the GRASS(Generally Regarded as safe substances).

RECOMMENDATION

The study has shown that action bitters elicit quite a number of toxic effect on animals and by extrapolation in man. A closer look at a pack of action bitters shows that it contains up to 42% of alcohol. Continued consumption of action bitters may not be beneficial to health.

REFERENCES

1. Dacie, l.V. and Lewis, M.S. Practical heamatology (Third Ed.). London: Churchill, Livingstone, 1991; 227-257.
2. Jain, N. C. (1986). Schalm's Veterinary Pharmacology. LEA and Febiger, 2826. Philadelphia.
3. Lorke, D. A new Approach to practical Acute Toxicity Testing. Archieves of Toxicology, 1983; 54: 275-287.
4. Ogbonnia S., Adekunle., A. A., Bosa M. K and Enwuru V. N. Evaluation of acute and subacute toxicity of Alstonia cogensis Engler (Apocynaceae) bark and Xylopia aethiopica (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. Afr J Biotechnol, 2008; 7(6): 701-705.
5. Onwusonye, J.C., Uwakwe, A.A., Iwuanyanwu, P and Muritala, I.K. Oral acute toxicity study of methano leaf extracts of Croton Zambesicus in mice. International Journal of Innovative Research Development, 2014; 3(7): 227-229.
6. Pieme C. A., Penlap V. N., Nkegoum B., Taziebou C. L., Tekwli, E. M., Etoa, F. X and Ngongang, J. Evaluation of acute toxicities, of aqueous ethanolic extracts of leaves of (L) Roxb (Ceasalpiniaceae). Afr J BiotechnoI, 2006; 3: 283-289
7. Rickert K., Martinez R. R and Martinez T. T Pharmacist Knowledge of Common Herbal preparations. Proc. West. Pharmacol. Soc., 1999; 42: 1-2.

8. Sushruta K., Satyanarayana S., Srinivas, N. and Sekhar, R. J. Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected Umbellifereous fruits used in culinary practice. Trap J Pharmaceutical Res., 2006; 5(2): 613-617.
9. Tedong L., Dzeufiet P. D. D., Dimo T., Asongalem E. A., Sokeng S. N., 1. F., Callard P and Kamtchouing P. Acute and Subchronic toxicity of Anacardium occidentale Linn (Anacardiaceae) leaves hexane extract in mice. Afr J of Traditional and Alternative medicine, 2007; 4(2): 140- 147.