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Acute and Sub-Chronic Toxicity Screening of Chloroform Extract of *Ficus capensis* in Rats

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Abstract

Chloroform extract of leaves of *Ficus capensis* previously defatted with n-hexane, a subject of an on-going research on the pharmacological basis for its use in Igala folk medicine for the typhoid fever therapy, was investigated for its *in vivo* toxicity in albino rats. The chloroform extracts were obtained by cold maceration, first defatted using n-hexane. Fifty-six healthy albino rats (174 ± 24 g) were randomized into seven groups of 8 animals (4 males, 4 females) each for the acute toxicity study. Sixty healthy albino rats (183 ± 39 g) were randomized into six groups of 10 animals (5 males, 5 females) each for the sub-chronic toxicity study. One group served as control while the other groups were administered with graduated doses of the extract. Animals that became moribund during the study and animals that survived the acute test period were sacrificed and necropsied. After every 7 days, 1 male and 1 female from each group of the sub-chronic toxicity test were sacrificed and necropsied. The animal organs were observed for physical signs of abrasions. The activities of marker enzymes such as serum aspartate transaminase, serum alanine transaminase and serum alkaline phosphatase, and total protein were determined using kits. The PCV was determined using the haematocrit capillary method. The median lethal concentration of *Ficus capensis* on albino rats was >5000 mg/kg body weight. There were no obvious signs of pharmacotoxicity in treated and control animals. There were no visible signs of abrasions or morphological changes in the organs of the treated animals in comparison to the control animals. Although the values of biochemical parameters obtained from treated animals differed significantly ($p<0.05$) from that of the control animals, all the values were within reference range. These results suggest that defatted chloroform extract of leaves of *Ficus capensis* does not provoke toxic response in rat models and may not be acutely or sub-chronically toxic in albino rats.

Keywords: Ficus capensis; Acute toxicity; Sub-chronic toxicity; Igala; Folk mediciney

Introduction

Medicinal plants used in the treatments of various ailments in Nigeria are numerous, one of which is *Ficus capensis*. The plant, *Ficus capensis* (Moraceae), also known as *Ficus sur* is a spreading deciduous or evergreen tree commonly known as fig tree. *Ficus capensis* is a medium sized tree mainly found in the tropics and growing up to 6-9 metres high [1-4]. In Igala folk medicine, it is used for the treatment of several febrile ailments, infectious diseases and for boosting the immune system [5-6]. In other studies, in Nigeria, the plant has been reported to be used in the management of dysentery and wound dressing [7] circumcision, leprosy and epilepsy, rickets, infertility, gonorrhoea, edema, respiratory disorders and as an emollient [1,8].

Several chemical constituents of plant material are responsible for the medicinal properties of plants used by traditional medical practitioners. These chemical constituents may include alkaloids, tannins, steroids, flavonoids, terpenoids, lipids, complex carbohydrates, glycopeptides, peptides and amines, cyanogens, and inorganic ions among numerous others [9]. Some of these compounds may elicit toxic response, making them inherently dangerous when consumed [10]. This present study was therefore aimed at evaluating the acute and sub-chronic toxicity of defatted chloroform extract of leaves of *Ficus capensis* in rats, as a part of a wider study.

Materials and Method

The plant materials were collected from Anyigba, North-Central Nigeria. They were identified by the Biological Sciences Department, Kogi State University, Anyigba, Nigeria. The plant samples (leaves) were collected in bags and then washed to remove debris. They were air dried at room temperature for about two weeks. Then it is pulverized using high speed Creston grinder. The pulverised samples were stored in plastic containers in the open laboratory until they were required.

The animals used for the study, albino rats (*Rattus novergicus*) of either sex, were obtained from the Animal House of the Department of Biochemistry, Kogi State University, Anyigba. The animals used in this experiment were adult albino rats. All the animals were kept in the Animal House of the Department of Biochemistry, Kogi State University, Anyigba, and were fed on standard laboratory food and water ad libitum. All animals were handled humanely.

Extraction

To obtain the chloroform extract, the leaves were first defatted with n-hexane. The pulverised plant sample (1000 g) was macerated in five litres of n-hexane in a capped vessel for 24 hours. Thereafter, the macerate was filtered through Whatman No 1 filter paper using a Speedvac vacuum pump. The residue obtained from the filtration was collected, dried and macerated in 5 litres of chloroform for another 24 hours, the filtrate was then concentrated using a rotary evaporator and

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dried on a water bath to obtain the chloroform crude extract and the yield was determined relative to the starting material

Median Lethal Dose (Ld_{50})

The LD_{50} was carried out by the revised Up and Down procedure (UPD) (USEP, 1998). A single dose of 3000 mg/kg body weight was administered to 4 healthy albino rats p.o. If the mortality of more than two animals was observed, the dose was reduced, but if mortality observed was less than two animals the dose was increased to 5000 mg/kg body weight Thereafter, there would be no need to increase the dose.

Acute Toxicity Studies

Fifty-six healthy albino rats (174 ± 24 g) were randomized into seven groups of 8 animals (4 males, 4 females) each. Each animal in Group 1 was treated p.o. with a single dose of 800 mg/kg body weight of the crude extract in 5 ml of normal saline. Similarly, each animal in Groups 2, 3, 4, 5, and 6 were treated with 1200 mg/kg, 1600 mg/kg, 2000 mg/kg, 3000 mg/kg and 50000 mg/kg body weight of the crude extracts respectively. Group 7 animals, the control group received equal aliquot of normal saline, the control vehicle. The animals were observed for clinical signs, morbidity and mortality at regular interval of 4 hours on the first day and thereafter daily for 14 days. Animals that became moribund during the study were sacrificed by chloroform anaesthesia and necropsied. Animals that survived the test period were sacrificed by chloroform anaesthesia and necropsied. The following organs; the heart, liver, kidney and lung were observed for physical signs of abrasions. Blood samples were obtained by cardiac puncture and collected in plain test tubes. The packed cell volume (PCV) of the animals was determined by the haematocrit capillary method. The serum samples were obtained by centrifuging the blood samples at 3000 rpm for 10 minutes, and stored at -20°C until used. The activities of marker enzymes serum aspartate transaminase (AST), serum alanine transaminase (ALT) and serum alkaline phosphatase (ALP) were assayed using Dialab ready to use kits and the total protein was determined using the Randox protein kit.

Sub-chronic Toxicity Studies

Sixty healthy albino rats (183 ± 39 g) were randomized into six groups of 10 animals (5 males, 5 females) each. Each animal in Group 1 was treated with 100 mg/kg body weight of the crude extract in 5 ml of normal saline administered orally daily for 28 days. Similarly, each animal in Groups 2, 3, 4 and 5 respectively was treated with 400 mg/kg, 800 mg/kg, 1200 mg/kg and 2000 mg/kg body weight of the crude extracts for 28 days. Group 6 animals that served as control received equal aliquot of normal saline, the control vehicle, for the same duration. The animals were observed for clinical signs, morbidity and mortality at regular interval of 4 hours on the first day and thereafter daily. After 7 days, 1 male and 1 female from each group were sacrificed by chloroform anaesthesia and necropsied and the following organs; the heart, liver, kidney and lung were observed for physical signs of abrasions. The blood samples were obtained by cardiac puncture and collected into plain test tubes. The PCV was determined using the haematocrit capillary method. The serum samples were obtained by centrifuging the blood samples at 3000 rpm for 10 minutes, and stored at -20°C until used. The activities of marker enzymes; serum AST, serum ALT and serum ALP were assayed using Dialab ready to use kits and the total protein was determined using the Randox protein kit.

The same sets of experiments were repeated with 1 male and 1 female from each group after 14, 21 and 28 days of administration respectively. The administration of oral dose of the test substance was stopped after 28 days. Thereafter, the same sets of experiment were repeated on all surviving animals by the 35th day (7 days after administration of test substances were stopped).

Statistical Analysis

The data obtained were presented as mean ± standard error of mean (SEM) of three replicate determinations and analysed by Statistical Package for the Social Sciences v16 (SPSS Inc. Chicago, USA). Student t-test was used to determine differences between the means of parameters in the respective test and control groups, analysis of variance (ANOVA) was used to determine the differences in mean between and within groups. P-values<0.05 were accepted as significant.

Result

LD_{50}

After administering a single dose p.o. of up to 3000 mg/kg body weight of the extract of *Ficus capensis* to four healthy albino rats, no death was observed after twenty-four hours. The dose of test substance administered to the test organisms was increased to 5000 mg/kg body weight and no death was observed after twenty-four hours. Thus LD_{50} of *Ficus capensis* on albino rats was greater than 5000 mg/kg body weight.

Acute toxicity of extract on *Rattus novergicus*

There were no observable signs of pharmacotoxicity such as ataxia, vomiting, listlessness, tremor, piloerection and difficulty in breathing in animals that received p.o. doses of *Ficus capensis* and the animals that served as control in the duration of the test. There were also no changes in the skin colour, fur and eyes in the test period. However, there was loss of appetite observed in animals that were treated with p.o. doses of extracts of *Ficus capensis* from 12 hours post administration to about 24 hours. This was not observed in the animals that received only normal saline. Mortality check revealed that on the tenth day post administration, one animal died from the group of female that received normal saline but no death occurred in the animals treated with extracts of *Ficus capensis*. The animals gained weight over the test period. The weight changes were random but the treated animals had a significantly ($p<0.05$) lower weight gain compared to the control animals.

The surviving test animals were sacrificed on the 15th day by chloroform anaesthesia. The liver, heart, kidney and lungs were removed and physically inspected for signs of lesion and changes in the morphology of the organs and comparing them to those of the control animals. There were no physical signs of abrasions or changes in morphology in the organs of the treated animals compared to the control animals. Isolated cases of lesion of some organs were however observed, but this was not consistent in a treated group.

The PCV of the male control animals was 56% while that of the female was 40%. For animals that were treated with *Ficus capensis*, their PCV ranged from 48% to 52% for male animals and 33%-38% for female animals. These values are significantly ($p<0.05$) lower than those of the controls. Serum Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and the total protein of the treated animals and control were determined. Table 1 shows that the values of the parameters for the male and female rats treated with chloroform extract of *Ficus capensis* all fell within the reference range.

Sub-chronic toxicity of extract on *Rattus novergicus*

From the seventh day, immediately the treated animals received their daily dose, they would be restless for about ten minutes, thereafter, lethargy would set in for about one hour, after which they would resume their normal activities. During the period of observation post treatment, there was no sign of restlessness and lethargy in the animals. Loss of

Sex	Treatment (mg/kg b. wt.)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Protein (g/L)
Male	Normal saline	8.23	10.32	113.71	66.4
	800	8.32	10.37	116.73	64.8
	1200	8.23	10.44	117.53	66.2
	1600	8.27	10.46	119.26	64.7
	2000	8.33	10.47	113.95	65.4
	3000	8.35	10.47	114.07	64.1
	5000	8.43	10.41	118.75	64.6
Female	Normal saline	7.92	10.21	85.03	66.9
	800	7.96	10.36	88.89	65.4
	1200	7.94	10.31	89.13	66.2
	1600	8.01	10.32	89.31	64.2
	2000	8.17	10.29	86.81	65.7
	3000	7.98	10.31	85.39	64.8
	5000	8.06	10.34	89.94	64.3
Reference values		<12	<12	73-207	64-83

Table 1: Effect of acute toxicity of the crude chloroform extract of *Ficus capensis* on the biochemical parameters of male and female rats treated p.o with a single dose

appetite and progressive weight loss was also apparent in the treated animal; these were however reversed in the period post treatment. Aside this no serious pharmacotoxic sign was observed in the treated animals. Mortality check revealed that one female animal that received normal saline from the control group died on the eighteenth day. On the twenty first day, a female animal treated p.o. with 800 mg/kg body weight. of the extract died. On the twenty sixth day one male and one female in the groups treated p.o. with 2000 mg/kg body weight also died.

There was a significant ($p<0.05$) decrease in the PCV values of the animals treated with the test substances compared to the control animals. The decrease was not dose dependent but was time dependent. There was an apparent increase in the PCV value post treatment as shown in Table 2.

Tables 3-6 shows the values of biochemical parameters such as AST, ALT, Alkaline phosphatase and total protein of treated and control animals. The values for animals treated with chloroform extract of *Ficus capensis* were significantly different from the values of the control animals, but the values for the treated animals and the controls were within the reference range.

Discussion

Three treated animals died in the period of the experimental study, but it cannot be concluded that the extracts administered were responsible for the deaths because two of the animals from the control group also died. Therefore, other reasons such as handling of the animals may be adduced for the cause of death. This assertion may be supported by the result of the LD50. A median lethal dose of >5000 mg/kg body weight was observed for the extract. Since it is a herbal extract and herbal formulations with LD50 above 3000 mg/kg/oral may be regarded as safe for consumption [11] the extract could be regarded as not acutely toxic.

The first signs of toxicity include changes in body weight, and general behaviors, and thus critical for the objective assessment of the effect of a compound on test animals [12]. There were no apparent noxious signs in the animals treated with the extracts of *Ficus capensis*. This may suggest that the extract does not provoke acute toxic response in the treated animals. Nonetheless, the reduced weight gain observed in the treated animals compared to the controls for the acute studies, and the progressive loss of weight observed in the treated animals in

Sex	Treatment (mg/kg b. wt.)	PCV (%) After Treatment				
		7 Days	14 Days	21 Days	28 Days	7 Days Post Treatment
Male	Normal saline	54	56	56	56	56
	100	51	49	47	46	48
	400	53	48	46	44	45
	800	50	48	45	43	44
	1200	49	47	43	43	44
	2000	43	41	40	38	40
Female	Normal saline	47	40	44	45	46
	100	36	36	34	31	36
	400	35	34	33	31	33
	800	35	36	32	30	34
	1200	40	37	34	32	36
	2000	38	38	33	30	34

Table 2: Effect of sub-chronic toxicity of the crude chloroform extract of *Ficus capensis* on PCV of rats treated with sub-chronic oral doses.

Sex	Treatment (mg/kg b. wt)	AST (U/L) after days of treatment				
		7 Days	14 Days	21 Days	28 Days	7 Days Post Treatment
Male	Control	8.34	7.67	8.3	8.39	8.56
	100	8.41	8.44	8.58	8.61	8.59
	400	8.53	8.57	8.59	8.67	8.6
	800	7.62	8.12	8.49	8.72	8.65
	1200	9.03	9.07	8.99	9.21	8.97
	2000	8.33	8.57	9.02	9.12	8.82
Female	Control	9.12	9.32	9.21	9.22	9.3
	100	6.03	6.39	6.76	7.34	7.32
	400	7.32	7.45	7.65	7.82	7.63
	800	8.76	8.86	8.91	8.98	8.89
	1200	9.87	9.88	10.01	10.21	10.17
	2000	9.54	9.61	10.01	10.02	10.09
Standard		<12				

Table 3: Effect of sub-chronic toxicity of the crude chloroform extract of *Ficus capensis* on AST of rats treated with sub-chronic oral doses.

Sex	Treatment (mg/kg b. wt)	ALT (U/L) after days of treatment				
		7 Days	14 Days	21 Days	28 Days	7 Days Post Treatment
Male	Control	10.22	10.1	10.26	10.32	10.28
	100	9.92	10.1	10.36	10.38	10.35
	400	10.12	9.97	10.36	10.47	10.38
	800	10.03	10.37	10.47	1.48	10.4
	1200	10.23	10.38	10.52	10.56	10.51
	2000	10.24	10.36	10.6	10.65	10.61
Female	Control	10.31	10.31	10.34	10.43	10.42
	100	10.02	10.25	10.57	10.61	10.55
	400	10.26	10.31	10.62	10.75	10.68
	800	10.38	10.46	10.72	10.81	10.79
	1200	10.51	10.36	10.86	10.96	10.92
	2000	10.54	10.59	11.02	11.23	10.98
Reference value		<12				

Table 4: Effect of sub-chronic toxicity of the crude chloroform extract of *Ficus capensis* on ALT of rats treated with sub-chronic oral doses.

Sex	Treatment (mg/kg b. wt)	ALP (U/L) after days of treatment				
		7 Days	14 Days	21 Days	28 Days	7 Days Post Treatment
Male	Control	112.51	112.21	113.45	112.64	113.09
	100	114.26	115.38	116.19	119.03	119.05
	400	115.32	116.22	117.84	119.67	119.23
	800	118.32	121.98	122.23	122.87	121.98
	1200	117.94	126.33	125.2	129.07	128.67
	2000	118.44	124.16	126.19	128.43	128.08
Female	Control	83.08	81.23	84.97	83.68	83.56
	100	95.9	96.07	96.34	96.51	96.45
	400	95.32	96.25	96.37	96.48	96.42
	800	94.07	96.51	96.43	98.12	97.89
	1200	88.51	91.23	93.07	97.89	97.31
	2000	96.49	110.01	110.37	112.09	112.021
Reference range		73-207				

Table 5: Effect of sub-chronic toxicity of the crude chloroform extract of *Ficus capensis* on ALP of rats treated with sub-chronic oral doses.

Sex	Treatment (mg/kg b. wt)	Total Protein (g/L) after days of treatment				
		7 Days	14 Days	21 Days	28 Days	7 Days Post Treatment
Male	Control	66.4	66.4	66.7	66.2	67
	100	66.2	65.8	65.6	64.9	65.6
	400	66.2	65.3	65.1	64.5	65.3
	800	66.1	64.8	64.7	64.4	65.4
	1200	66.3	64.2	64.1	63.8	65.2
	2000	65.6	64	64.1	63.6	64.8
Female	Control	66.8	66.9	66.7	66.7	66.6
	100	66.3	66.2	65.5	65.1	65.7
	400	66.1	65.8	65.3	64.9	65.2
	800	65.8	66.1	65.7	65.3	66
	1200	66.7	65.6	65.1	64.8	65.3
	2000	65.9	65.4	65.1	64.2	65.2
Reference range		64-83				

Table 6: Effect of sub-chronic toxicity of the crude chloroform extract of *Ficus capensis* on Total Protein of rats treated with sub-chronic oral doses.

the sub-chronic studies unlike the control group may be explained by the loss of appetite observed in the treated animals. However, the effect was reversible post treatment suggesting that the actions of the extract could be reversible when treatment is discontinued.

Usually, the major toxic effect involves one or two organs and they represent target organs of toxicity of the particular substance [13]. The absence of macroscopic evidence of abrasion in the liver, heart, lung and kidney of the test animals or change in colour of the organs of the treated animals compared to the control animals imply that the extract may not be toxic to these organs.

Furthermore, blood chemistry parameters which could potentially to ascertain damage to liver, kidney, heart and other internal organs [14] showed that animals treated p.o. with the extracts of *Ficus capensis* in the acute toxicity studies had values within the reference range. Results obtained from the sub chronic toxicity test indicated that there was an elevation of biochemical markers such as AST, ALP ALT of animals treated with the extract with increasing length of treatment, but not in a concentration dependent manner. Conversely, the total protein of treated animals declined over the treatment period. These trends were however reversed seven days post treatment, and all the values still fell within the reference range. These results suggest that extract of *Ficus capensis* may not be toxic to rats and any adverse effect elicited by the administration of the extract is reversible.

Bone marrow, the major site of production of blood cells, is one of the locations where the adverse effects of test substances are elicited. Therefore, any compound that affects the bone marrow could prevent the generation of haemoglobin in red blood cells, and then reduce the ability of the blood to distribute oxygen throughout the body [15]. The PCV values of the male and female rats treated with the extracts were significantly lower than the control values. The variation did not follow any pattern, as it was not dose dependent. However, the alterations in PCV value were still within the reference range. This could indicate that the extract does not have toxic effects on the bone marrow of rats.

Conclusion

Based on these results obtained from the study, it may be concluded that defatted chloroform extract of Ficus capensis does not provoke toxic response in albino rats and may not be toxic in rats.

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