Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 10 (4): 173-179, 2015
ISSN 1815-9923 / DOI: 10.3923/ajb.2015.173.179

Phytochemicals and Acute Toxicity Profile of Aqueous and Methanolic Extracts of *Crateva adansonii* Leaves in Swiss Albino Rats

[1]Tsado N. Amos, [2]Lawal Bashir, [1]Santali E. Saba, [1]Mohammed A. Saba, [1]Balarabe M. Mohammed, [1]Ibrahim H. Abdulsalam and [1]George J. Josiah
[1]Department of Basic and Applied Sciences, Niger State Polythechnic Zungeru, Niger, Nigeria
[2]Department of Biochemistry, Federal University of Technology, PMB 65, Minna, Niger, Nigeria

Corresponding Author: Tsado N. Amos, Department of Basic and Applied Sciences, Niger State Polythechnic Zungeru, Niger, Nigeria

ABSTRACT

Acute toxicity and phytochemical studies of aqueous and methanolic leaf extract of *Crateva adansonii* were investigated. The phytochemical screening and acute toxicity studies were conducted using standard procedure and methods. The weights of the rat were monitored before and after the experiment. The phytochemical analyses of the aqueous and methanolic leaf extract of *Crateva adansonii* leaf showed the presence of alkaloids tannins, flavonoids, saponins cardiac glycoside and steroids in both aqueous and methanolic leaf extract of *Crateva adansonii*. Phlobatannins were absent in aqueous but present in methanolic leaf extract of *Crateva adansonii*, while anthraquinones were not detected. The results of the weight changes show that there is no statistically significant ($p>0.05$) difference in weight gain or weight loss of rat administered with aqueous and methanolic leaf extract of *Crateva adansonii* as compared with the control group and the LD_{50} of the aqueous and methanolic leaf extract in rat was found to be greater than 5000 mg kg^{-1} body weight. It is concluded that *Crateva adansonii* contain active phytochemicals of therapeutic potential and that the extract is safe for clinical application.

Key words: Phytochemicals, *Crateva adansonii*, acute toxicity

INTRODUCTION

Nature has presented to humanity the gift of vast therapeutic workshop with wide varieties of medicinal plant. There are over 250,000 species of flowering plant on earth, 155,000 of which are found in the tropics (Cordell, 2000). According to WHO more than 80% of world's population, are thought to depend chiefly on traditional medicine, which is largely of plant origin, for their primary health care needs (WHO., 2010). However, it is widely believed that this valuable medicinal resource in plants is largely untapped because of inadequate scientific technical and commercial infrastructures in developing countries (Akerelo, 1993).

In recent years, there is a growing interest in herbal therapy. Data on scientific screening of plant extracts, whether crude or purified, appears to be accumulating gradually but steadily. The major contributory factors to this growing interest include: Rising costs of orthodox medications, low therapeutic index of synthetic compounds and the growing incidence of drug resistance among the pathogens especially in developing countries with very weak economic indices. It is thought

that the use of plant-derived active principles will offer people access to safe and effective products for the prevention and treatment of diseases through self-medication. One major and over riding criterion in the selection of herbal medicines for use in health services is safety. Plants extracts should not only be efficacious but safe for consumption. Therefore, closely associated with screening of plants extracts for their activities against microorganism or disease conditions is the need to know their toxic potentials (Fleming and Hunt, 2000).

Crateva adansonii DC, also known as *Crateva religiosa* or sacred garlic pear, belongs to family Capparaceae is in high demand, especially its leaves for the treatment of ear infections (Tsado *et al*., 2015). The bark is widely used for stomach troubles and held to have tonic properties. In Senegal the roots figure in several treatments for syphilis, jaundice and yellow. Ayodeji *et al*. (2011) claimed the antimicrobial properties of its leaves. Two phytoconstituents had also been isolated and identified as oleanolic acid and 4-epi-hederagenin. By the comparative analysis, it is find that oleanolic acid have less (LC_{50} 2.51 μg mL^{-1}) as compared to the standard cycloheximide (LC_{50} is 40 μg mL^{-1}) (Cantrell *et al*., 2003). Despite the therapeutic potential of these plant toxic properties have also been attributed to the plant especially the leaves and seed. Various organic and aqueous extract of *Crateva adansonii* exhibited different toxic symptom depending on the dose, mode of administration and sensitivity of animal being tested. The present study, therefore, aimed at evaluating the phytochemicals and acute toxicity profile of aqueous and methanolic extracts of *Crateva adansonii* leaves in swiss albino.

MATERIALS AND METHODS

Plant collection: The leaves of *Crateva adansonii* was collected in August, 2014 from a tree in Zungeru, Niger State, Nigeria. Taxonomic authentication of the plant was conducted in the Department of Biology, Federal University of technology, Minna, Niger State, Nigeria.

Experimental animal: Swiss adult albino rat weighing between 130-155 g were bought from animals breeding unit of the Department of Biochemistry, University of Ibadan, Oyo State. They were housed in plastic cages and given standard laboratory diet and water *ad libitum* and are maintained under standard laboratory conditions.

Sample preparation and extraction procedure: The collected fresh leaves of *Crateva adansonii* were destalked, washed with clean-water, dried at room temperature and finally grounded using a grinder mill. Exactly 200 g of the dried sample was ground and boiled in 1 L of distilled water contained in a conical flask for 1 h. The extract was thereafter filtered hot first with muslin cloth and then with filter paper. Another 200 g of the grounded samples, were placed in conical flask contain 200 mL of 95% methanol. The mixture were refluxed for 60 min, the extracts were filtered through Whatman filter paper No. 1. The filtrates was concentrated in a water bath with its temperature set at 50°C for 2 days. The concentrated extract was finally exposed to air to complete drying. The dried extract was stored in a refrigerator at 4°C until required.

Phytochemical analysis

Test for glycoside: *Crateva adansonii* extracts (0.2 g) were mixed with 2 mL of glacial acetate and 1 drop of ferric chloride solution, after which 1 mL of concentrated sulphuric acid were added. The reaction was observed for a brown ring formation.

Test for steroids: *Crateva adansonii* extracts (0.2 g) were mixed with 2 mL of acetic anhydride followed by 2 mL of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for flavonoids: *Crateva adansonii* extracts (0.2 g) were heated with 10 mL of ethyl acetate in a test tube over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. Yellow coloration was observed that indicated the presence of Flavonoids (Harborne, 1973).

Test for tannins: *Crateva adansonii* extracts (0.5 g) were boiled in 20 mL of distilled water in a test tube and filtered. The 0.1% ferric chloride ($FeCl_3$) solution was added to the filtrate. The appearance of brownish green or a blue-black colouration indicates the presence of tannins in the test samples (Harborne, 1973).

Test for saponins: *Crateva adansonii* extracts (2.0 g) were boiled in 20 mL of distilled water in a test tube in boiling water bath and filtered. Ten milliliter of the filtrate was mixed with 5 mL of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion characteristic of saponins.

Test for anthraquinones: *Crateva adansonii* extracts (0.5 g) were shaken with 5 mL of chloroform. The chloroform layer was filtered and 5.0 cm^3 of 10% ammonia solution was added to the filtrate. The mixture was shaken thoroughly and the formation of a pink/violet or red, yellow colour in the ammoniacal phase indicates the presence of anthraquinones (Harborne, 1973).

Test for alkaloids: *Crateva adansonii* extracts (0.5 g) were stirred with 5 cm^3 of 1% aqueous HCl on a steam bath. Few drops of picric acid solution was added to 2 cm^3 of the extract. The formation of a reddish brown precipitate was taken as a preliminary evidence for the presence of alkaloids (Harborne, 1973; Trease and Evans, 1989).

Test for phlobatannins: *Crateva adansonii* extracts (2 g) were boiled with 1% aqueous hydrochloric acid, the formation of red precipitate thus indicated the presence of phlobatanins (Harborne, 1973).

Experimental design for acute toxicity study: The acute toxicity study was conducted in accordance with Lorke's method (Lorke, 1983). The acute toxicity study was conducted to observe the range of toxicity so the proper dose level could be established. The study were conducted in two phase. In the first phase nine rats were dividing into 3 groups of 3 rats each. Group 1, 2 and 3 animals were given 10, 500 and 1000 mg kg^{-1} b.wt. of the extract, respectively to possibly established the range of dose producing any toxic effect, in addition a fourth group of 3 rates were set up as control group.

In the second phase, the experiment was set up like the first phase but with the oral administration of 1600, 2900 and 5000 mg kg^{-1} b.wt. dose of the extract to group 1, 2 and 3,

respectively. All the above mention procedure was carried out for the aqueous and methanolic extract of *Crateva adansonii*.

The extract was dissolved in Phosphate Buffered Saline (PBS) solution and given via intraperitoneal route. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity.

RESULTS

Phytochemical composition: Table 1 show the result of qualitative phytochemical composition of aqueous and methanolic leaf extract of *Crateva adansonii*. The results revealed, the presence of Alkaloids tannis, flavonoids, saponins cardiac glycoside and steroids in both aqueous and methanolic leaf extract of *Crateva adansonii.* However, phlobatannins were absent in aqueous but present in methanolic leaf extract of *Crateva adansonii*, while, anthraquinones was completely absent in both aqueous and methanolic leaf extract of *Crateva adansonii*.

Acute toxicity (LD_{50}): The LD_{50} of Methanol and aqueous extract of *Crateva adansonii* was found to be more than 5000 mg kg^{-1} b.wt. No death or sign of toxicity were recorded within 24 h after treatment with the extract and also no death was recorded among all the dose groups throughout the two weeks experimental period. Mice at dose of 5000 mg kg^{-1} b.wt. show a behavior changes of rubbing of nose and mouth on the floor of the cage. This however, disappear within 24 h of extract administration (Table 2).

Weight changes (G): Table 3 and 4 show the result of weight changes in rat following the administration of aqueous and methanolic leaf extract of *Crateva adansonii*. There exist no

Table 1: Phytochemical composition of aqueous and methanol extract of *Crateva adansonii*

Phytochemicals	Aqueous extract	Methanol extract
Tannins	++	+
Phlobatannin	-	+
Saponin	+	+
Flavonoid	+	+
Steroid	+	+
Terpenoid	+++	+
Alkaloid	++	++
Anthraquinone	-	-
Cardiac glycoside	+	+

-: Absent, +: Present, ++: Significant, +++: Very significant

Table 2: Acute toxicity effect of aqueous and methanol leaf extract of *Crateva adansonii*

Groups	Dosage (mg kg^{-1})	Mice 1	Mice 2	Mice 3
Phase 1				
Group 1	100	ND and NST	ND and NST	ND and NST
Group 2	500	ND and NST	ND and NST	ND and NST
Group 3	1000	ND and NST	ND and NST	ND and NST
Group 4	Control	ND and NST	ND and NST	ND and NST
Phase 2				
Group 1	1600	ND and NST		
Group 2	2900	ND and NST		
Group 3	5000	ND		

ND: No death, NST: No sign of toxicity

Table 3: Effect of methanol leaf extract of *Crateva adansonii* administration on weight changes of rat

		Weight changes (g)		
Groups	Dosage (mg kg^{-1})	Day 0	Day 14	Weight gain
Phase 1				
Group 1	10	160.0±11.2	193.2±7.11	33.2±4.00[a]
Group 2	100	172.0±9.0	201.7±12.70	29.7±4.21[a]
Group 3	1000	168.5±3.8	199.4±11.11	30.9±5.35[a]
Group 4	Control	167.9±7.4	199.2±9.70	31.3±4.76[a]
Phase 2				
Group 1	1600	172.9	198.07	26.17
Group 2	2900	165.5	196.72	31.22
Group 3	5000	169.60	192.41	22.81

Data are Mean±SEM of triplicate determination, Value followed by the same superscript are not significant different at p<0.05

Table 4: Effect of aqueous leaf extract of *Crateva adansonii* administration on weight changes of rat

		Weight changes (g)		
Groups	Dosage (mg kg^{-1})	Day 0	Day 14	Weight gain
Phase 1				
Group 1	10	1650.0±11.2	194.2±7.11	29.2±5.00[a]
Group 2	100	177.0±9.0	206.7±13.70	29.7±4.01[a]
Group 3	1000	175.4±3.8	287.3±9.09	31.9±5.25[a]
Group 4	Control	170.9±7.4	204.2±9.70	33.3±2.76[a]
Phase 2				
Group 1	1600	169.9	200.07	30.17
Group 2	2900	170.5	199.72	29.22
Group 3	5000	172.60	200.41	27.81

Data are Mean±SEM of triplicate determination, Value followed by the same superscript are not significant different at p<0.05

statistically significant (p>0.05) difference in weight gain or weight loss of rat administered with aqueous and methanolic leaf extract of *Crateva adansonii* as compared with the control group.

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agent. Phytochemical component are responsible for both pharmacological and toxic activities of plant (Lawal *et al*., 2005). Plant are known to produce this compound to protect them self against predators but studies show that they can also be use to protect human against disease. This study revealed the presence of various medicinal important phytochemicals including, Terpenoids, saponins, glycosides, alkaloids and flavonoids in methanolic and aqueous extract of *Crateva adansonii*.

Flavonoid have been reported for their anti-mutagenic anticarcinogenic potentials due to their antioxidant and anti inflammatory properties. Saponin are use as adjuvant in the production of vaccines (Asl and Hosseinzadeh, 2008). Steroids are used in the stimulation of bone marrow and growth. It stimulates lean body mass and also play vital roles in the prevention of bone loss in elderly men. Alkaloid has been used as CNS stimulant, topical anesthetic in ophthalmology, powerful painkillers, antipyretic action among other use. The cardiac glycoside has been used for over two centuries as stimulant in cases of cardiac failure and diseases. The presence of tannin in the leaf extract of *Crateva adansonii* suggests the ability of these plants to play major roles as antifungal antidiarrheal, antioxidant and antihemorrhoidal agent (Asquith and Butler, 1986). Tannin also have astringent property, plant containing tannin has been reported to be used for healing of wounds, varicose ulcers, hemorrhoids, frostbile and burn in herbal medicine (Igboko, 1983).

The presence of all this phytochemcials in the leaf extract of *Crateva adansonii* is an indication that this plant if properly screened could yield a drug of pharmacological significant. However, the absence of phlobatannins in methanolic extract but present in aqueous extract and the absence of anthraquinone in both extract agree with early studies which also found that not all phytochemicals are present in all plant and those present differ with the solvent use in the extraction process (Tijjani *et al*., 2007).

The acute lethal effect of *Crateva adansonii* show that no animal died throughout the period of the experiment at all dose of the extract. These show that the LD_{50} is greater than 5000 mg kg^{-1} b.wt. although, there is behavioral change (rubbing of the eye and mouth on the floor of the cage) observer at dose level of 5000 mg kg^{-1}. These effects however, disappear within the 48 h of extract administration. The LD_{50} been greater than 5000 mg kg^{-1} b.wt. is through to be safe as suggested by Lorke (1983). Again, the absence of death among rats in all the dose groups throughout the two weeks of the experimental goes further to support this claim.

Furthermore, the administration of the extract did not show any statistically significant ($p>0.05$) weight gain or weight loss as compared with the control group this finding is an indication of the safety of *Crateva adansonii* for consumption and clinical application.

CONCLUSION

From this study it is hypothesized that the aqueous and methanolic extract of *Crateva adansonii* contain active phytochemicals of therapeutic potential and that the extract is safe for clinical application as the LD_{50} is greater than 5000 mg kg^{-1} b.wt.

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