

## **Phytochemical screening and toxicological implication of administration of aqueous cocoa powder extract obtained from Nigeria in albino rat**

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### **ABSTRACT**

*The therapeutic use and toxicological implication of plant materials is dependent upon the different phytochemicals present and thus safety of its consumption by animals and human. Aqueous cocoa powder extract was subjected to phytochemical screening and toxicological (acute and chronic) studies using female albino rats. Acute toxicity study was done using twenty five rats (divided into five groups of five rats each). Chronic toxicity study was done using different doses (200mg/kg, 300mg/kg, 500mg/kg, and 1000mg/kg) of aqueous cocoa powder extract administered orally to forty female albino rats (divided into five groups of eight rats each) continuously for 40 days while the control group was given rat chow and water only. LD<sub>50</sub> was determined after oral administration of a single dose of the extract while plasma urea; creatinine; aspartate transaminase and alanine transaminase activities were measured to determine nephrotoxic and hepatotoxic effect of the extract. The result of the phytochemical screening showed that the extract is abundantly rich in flavonoids, alkaloids and terpenoids; appreciably rich in cardiac glycoside, anthraquinone glycoside, tannin, and phenol; rich in saponin but lack cardenolide. The LD<sub>50</sub> value was found to be above 5000mg/kg and the plasma urea, creatinine, aspartate transaminase and alanine transaminase activities were found to be within normal range, though with mixed level of significance among the administered doses in comparison to the controls. Short and long term administration of aqueous cocoa powder extract is thus non-toxic as it may not potentiate renal and hepatic toxicity and may be considered safe for animal consumption.*

**Key words:** Cocoa powder, phytochemicals, toxicology, LD<sub>50</sub>, transaminases

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### **INTRODUCTION**

Globally, the trend in the management of chronic diseases is driving towards modifying existing knowledge of the use of plant materials, discovering new activities in plant materials and removing the taboos associated with the use of such plant materials especially in Africa, notably Nigeria. This global change is consequent to the development of multidrug resistance, non-drug compliance by patients, development of secondary complications and side effects associated with the chronic use of some drugs in disease management. Africa is rich in such future promising and hopeful solutions to these problems considering the vast number of discovered and yet to be discovered or classified

plant species available in the continent. This simple cost-effective solution will improve human health and prevent disease progressions thus having a profound beneficial impact.

About eighty per cent of the world population particularly in the developing countries depends to a large extent on traditional medicines mainly derived from plant sources for the management of chronic diseases [1, 2]. This is the main reason why research is being intensified to identify as many plant species as possible with potential therapeutic values [3, 2]. With modern medical practices, there is the need to compare efficacy of identified plant materials with existing standard drugs using scientific and clinical data and probably amalgamate them for their synergistic effects.

There is thus a need for conducting research in herbal drugs, developing simple bioassays for biological standardization; pharmacological and toxicological evaluation; and developing various animal models for toxicity and safety evaluation. It is also important to establish the active component(s) from these plant extracts. Many traditional medicine in use are derived from medicinal plants, minerals and organic matter [4].

Among the 21,000 plants listed by WHO that are used for medicinal purposes around the world, 2500 species are in India, out of which 150 species are used commercially on a fairly large scale [2]. India is the largest producer of medicinal herbs and is referred to as botanical garden of the world [5].

The current research focuses on the phytochemical screening and determination of toxicological implication of the use of aqueous cocoa powder extract in order to predict its medicinal value and safety for consumption.

## **MATERIALS AND METHODS**

### **Source of cocoa powder**

Pure cocoa powder was obtained from appetizing food company, Ibadan, Nigeria and used for the study.

### **Phytochemical screening**

Phytochemical screening was performed using standard procedures described by Trease and Evans; and Sofowora [6, 7]. This include test for anthraquinones, terpenoids, flavonoids, saponins, tannins, alkaloids, cardenolide, phenols and cardiac glycosides.

### **Care of Animals**

The care of the animals was in accordance with the US Public Health Service Guidelines [8].

Environmental factors (such as space, light, noise, and temperature), that affects animal well-being were taken care of. Cages were made of wooden nontoxic materials and smooth surfaced; prevented animal escape and allow for cleaning, disinfecting and regular animal handling; prevented accidental entrapment of animals and free of projections that may injure the animals. Animal house is well ventilated, and relatively silent from noise.

### **Acute toxicity study**

Acute toxicity study was performed according to the World Health Organization guideline (WHO) and the organization of economic co-operation and development guideline for testing of chemicals (OECD using thirty female albino rats [2, 9]. Female rats were used because literature surveys of conventional LD<sub>50</sub> tests showed that there is little difference in sensitivity between the sexes but when differences are observed, females are generally slightly more sensitive than male [10]. The method of Lorke was employed in the estimation of acute oral median dose (LD<sub>50</sub>) [11]. The LD<sub>50</sub> was calculated using the method of maximum likelihood [12].

Five animals were used for each dose level (300, 500, 1000, 2000 and 5000 mg/kg body weight). The animals were fasted overnight (food withdrawn but not water) prior to dosing after which the animals were weighed to determine the fasted body weight. The administered doses were calculated according to the body weight. The cocoa powder was measured, dissolved in 1mls water and administered as a single dose, using oral cannula. After the administration of the aqueous cocoa powder, food was further withheld for about 4 hours.

The animals were group-caged according to the dose and observed individually for signs of toxicity after dosing; twice during the first 30 minutes, hourly during the first six hours, two hourly during the first 24 hours, and daily for a total of 14 days.

Toxicity signs given consideration include changes in skin and fur, eyes, respiratory and central nervous systems (tremors and convulsions); behavioral pattern, salivation, diarrhea, sleep and moribund condition. The control group also comprises of five rats but was given 1mls water instead of aqueous cocoa powder extract.

### Chronic toxicity study

Forty female Wistar strain albino rats were used to determine the chronic toxicological implication of aqueous cocoa powder extract. This was performed using renal and liver enzyme markers of toxicity as these are the main organs responsible for regulatory metabolism and waste excretion. Plasma urea, creatinine, aspartate transaminase and alanine transaminase levels were determined by methods described by Wybenga *et al.*, Henry *et al.* and Schumann and Klauke respectively [13, 14, 15].

### Data Analysis

Data analysis was done using SPSS version 17 statistical package. All variables were expressed as Mean and standard error of mean (Mean  $\pm$  SEM) and differences in mean between groups was done using ANOVA. Statistical significance level was set at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Table 1: Phytochemical screening of cocoa powder

Alkaloids	+++
Terpenoids	+++
Flavonoids	+++
Phenol	++
Cardiac glycosides	++
Anthraquinone glycoside	++
Tannin	++
Phylobatannin	++
Saponins	+
Cardenolide	-

Key:

+++ Abundantly present  
 ++ Appreciably present  
 + Present  
 --- Absent

Table 1 above shows the phytochemical screening of cocoa powder revealing the presence of alkaloids, cardiac glycosides anthraquinones, flavonoids, terpenoids, saponins, phylobotannin and tannins, while cardenolide was absent.

Table 2: The various doses of cocoa powder and acute toxicity

Dose (mg/kg)	Dose difference	Number of rats	Number of deaths	Mean death
Control (1 ml water)	0	5	0	0
300	300	5	0	0
500	200	5	0	0
1000	500	5	0	0
2000	1000	5	0	0
5000	3000	5	0	0

Table 2 above shows that no casualty was observed among the rats even at 5000mg/kg dose

### Calculation of LD<sub>50</sub>

LD<sub>50</sub> = Maximum dose - Y/ Number of rats per group

Y= sum of mean death

LD<sub>50</sub> = 5000 - 0/5  
 = 5000

Thus, LD<sub>50</sub> for cocoa powder is  $\geq 5000$

**Table 3: Summary of toxicity markers amongst the groups**

Dose/ Parameters	200mg/kg	300mg/kg	500mg/kg	1000mg/kg	Control
AST (IU/L)	45.8 ± 2.8	45.5 ± 1.9	50.4 ± 1.0 <sup>*</sup>	43.3 ± 2.7	42.0 ± 1.6
ALT (IU/L)	7.5 ± 2.3 <sup>*</sup>	9.0 ± 1.9 <sup>*</sup>	10.5 ± 2.0 <sup>*</sup>	13.3 ± 1.9 <sup>*</sup>	27.3 ± 4.0
Urea (mg/dl)	63.0 ± 2.6	73.5 ± 1.7 <sup>*</sup>	79.4 ± 5.2	75.0 ± 3.7 <sup>*</sup>	54.0 ± 3.2
Creatinine (mg/dl)	0.6 ± 0.2	0.8 ± 0.02	0.8 ± 0.02	0.7 ± 0.1	0.77 ± 0.21

Table 3 above shows the markers of renal and hepatic toxicity following aqueous cocoa powder extract administration in rat. Values were expressed as Mean ± SEM and levels of statistical significance was considered at  $p < 0.05$  as indicated by \*

The medicinal value and use of any plant materials is dependent upon the type, class, and amount of the different phytochemicals present and the method of extraction employed. The result of the phytochemical screening suggest that aqueous cocoa powder extract is abundantly rich in flavonoids, alkaloids and terpenoids; appreciably rich in cardiac glycoside, anthraquinone glycoside, tannin, phenol; rich in saponin and lack cardenolide (table 1).

Acute oral toxicity from any substance refers to those adverse effects that may occur following oral administration of a single dose of the substance, or multiple doses of the substance given within 24 hours while chronic toxicity is beyond this time frame. The toxicological evaluation of a plant material is usually done to determine the possible collateral effects of the plant materials and to ensure safety of its consumption by animals and human. This toxicity may be inherent of the herbal products or consequent of the method of extraction [16].

In interpreting results from toxicological studies, consideration must be given to the vehicle of administration as this may be the avenue for the observed toxicological signs. In this case, the toxicological signs may be from the vehicle and not the plant materials per se.

Investigation of the acute toxicity has been described by Lorke as the first step in the toxicological investigations of an unknown substance [10]. The index of acute toxicity is the LD<sub>50</sub>. The chemical labeling and classification of acute systemic toxicity of substances based on oral LD<sub>50</sub> values recommended by the Organization for Economic Co-operations and Development are as follow: very toxic ( $\leq 5$ mg/kg body weight), toxic ( $>5$  and  $\leq 50$ mg/kg body weight), harmful ( $>50$  and  $\leq 500$ mg/kg body weight), and no label ( $>500$  and  $\leq 2000$ mg/kg body weight) [8]. Any LD<sub>50</sub> values greater than 5000mg/kg body weight are usually considered to be of no practical interest [10]. From this study, the LD<sub>50</sub> value for aqueous cocoa powder extract was found to be above 5000mg/kg (table 2), thus aqueous cocoa powder extract is not toxic when administered by the oral route and may be considered safe for animal consumption.

LD<sub>50</sub> value is affected by some other factors such as animal species and strain, age, gender, diet, bedding, ambient temperature, caging conditions and time of the day; thus its extrapolation in man must be carefully done as the result will provide only a ballpark estimate of human lethality [17].

From the chronic toxicity study (table 3), it was observed that there was a majorly non-significant ( $p > 0.05$ ) increase in plasma AST levels while a significant ( $p < 0.05$ ) decrease in plasma ALT level was observed when compared with the control (table 3). Both AST and ALT are markers of liver toxicity with ALT much specific about measure of hepatocellular integrity.

Considering renal toxicity measures, a mixed significant increase in plasma urea concentration was observed (table 3), though the values are within the normal range [18]. Also, a non-significant ( $p > 0.05$ ) change in the plasma creatinine concentration was observed in the entire doses used (table 3).

## CONCLUSION

The LD<sub>50</sub> and chronic toxicity marker results obtained from this study suggest that acute and chronic administration of cocoa powder extract may not potentiate any significant toxic effect. Thus, its consumption and use for whatever purpose may be recommended. Also, the result of phytochemical screening suggests its potential medicinal value that needs to be further investigated and determined.

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