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Toxicological implications of crude alkaloidal fraction from *Cnestis ferruginea* D.C root on liver function indices of male Wistar rats

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ABSTRACT: The crude alkaloidal fraction of *Cnestis ferruginea* D.C root was evaluated for its effect on the liver function indices of Wistar rats. Prior to the evaluation, the LD_{50} of oral route was established which is 27 mg/kg body weight. For the sub-acute toxicity studies, albino rats were grouped into four (A-D). Groups A, B and C were orally administered with 3, 6 and 9 mg/kg body weight of the alkaloidal fraction on daily basis for 14 days while group D (the control) was treated like the test groups except that they received distilled water. The liver function indices were monitored progressively 24 h after 1, 7 and 14 daily doses of the alkaloidal fraction. The reduction ($P<0.05$) in the activities of liver alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in all the alkaloidal treated groups were accompanied by corresponding increase in the serum enzymes. Similar administration of the alkaloidal fractions increased the serum concentrations of albumin, conjugated and total bilirubin. The total protein in the serum and liver-body weight ratio decreased significantly. The histopathological examination revealed mild to severe disruption of the normal structural architecture of the liver characterized by the presence of red blood cells in the hepatocytes. The results suggest that alkaloidal fraction of *Cnestis ferruginea* D.C root has adverse effect on hepatic functions.

Keywords: *Cnestis ferruginea* D.C, alkaloidal fraction, liver functional indices

Introduction

The use of medicinal plants in the management of several ailments is increasing empirically probably due to the belief that they are harmless simply because they are 'natural'. They are also commonly used for self medication without supervision. This increase in popularity and the scarcity of scientific studies on the safety of these plants and their phytoconstituents have raised concerns regarding toxicity and adverse effects of these remedies (Gehlot, and Bohra, 2000; Saad *et al*., 2006). Therefore, there is the need to assess the toxic implications of traditionally used medicinal plants.

We have among these plants, *Cnestic ferruginea D.C* (Connaraceae), which posses medicinal and nutritional values. The plant is a perennial shrub found mainly in the savannah region of tropical West Africa. The plant is about 3.0-3.6m high. It is a wild, densely brown plant with pinnate leaves and brown fruits, which produces flowers within the months of January to March (Irvine, 1961).

Cnestic ferruginea has various therapeutic uses in herbal medicine. The extract from the roots is applied to the nostrils for migraines and sinusitis. A root decoction is taken as well known aphrodisiac and used as an enema by women with abortion and ovarian troubles (Irvine, 1961). The root is used for toothache and the powdered bark is

rubbed on the gums for pyorrhea among the Igbo tribe in Nigeria (Oliver, 1986; Okwu and Iroabuchi, 2004). The plant is an active ingredient in the decoction used presently by herbalists in Eastern Nigerian for treatment of gonorrhea, joint and waist pains, arthritis, rheumatism, stroke and syphilis (Okwu and Iroabuchi, 2004). Notwithstanding the previous phytochemical analysis and antimicrobial activity screening of the plant root extracts of the plant by Okwu and Iroabuchi (2004) where alkaloids among other phytochemicals was identified as a major phytoconstituent, there appears to be dearth of information on the toxicological implications of the alkaloid fraction from *Cnestic ferruginea* D.C root on the liver functional indices. Therefore, this work was intended to assess the toxicological effect of the alkaloidal fraction from *Cnestic ferruginea D.C* root on the liver functional indices of Wistar rats.

Materials and Methods

Plant materials

The roots of *Cnestic ferruginea D.C* was obtained from an uncultivated farmland in Umuahia, Nigeria and was authenticated at Department of Plant Biology, University of Nigeria, Nsukka, Nigeria where voucher specimen was deposited.

Animals

A total of 150 male, albino rats *(Rattus norvegicus)* of Wistar strain, weighing 160.10g $\pm$ 2.10g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Nigeria. The animals were housed in clean metabolic cages placed in well-ventilated house with optimum conditions (temperature: 28-31^{o}C; photoperiod: 12h/12h light/dark cycle; humidity: 50-55%). They were allowed free access to rat pellets (Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria) and tap water. The cages were cleaned daily.

Assay kits

The assay kits for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained from Randox Laboratories, Co-Atrim, United Kingdom. Other reagents used were of analytical grade and were prepared in glass-distilled water. The reagents were stored in bottles except for biuret which was stored in a plastic container (Plummer, 1978)

Preparation of the crude alkaloidal fraction (CAF)

The acid-base extraction method was employed for the preparation of the crude alkaloidal fraction. Briefly, the root was oven-dried at 40°C to a constant weight before being pulverized. Powdered *Cnestic ferruginea* root was extracted with 95% (v/v) MeOH at ambient temperature The MeOH extract was then concentrated under reduced pressure and acidified with 0.5M H_2SO_4. The acidic extract was washed with chloroform to remove neutral components. The aqueous acidic fraction was then made basic with ammonia (pH 10) and extracted again with chloroform until the aqueous layer was free of alkaloids. The combined chloroform extracts were evaporated in vacuo to yield the CAF as dark brown residue (0.27 (w/w) of the oven dried starting material). This was then reconstituted in distilled water to give the desired doses used for this experiment.

Acute oral toxicity studies

Graded doses of the CAF were given orally to 90 rats used for acute toxicity studies. Animal treatment and calculation of LD_{50} was performed according to the method previously used by Rujjanawate *et al* (2003).

Sub-acute toxicity studies

60 rats were completely randomized into four groups (A, B, C and D) of 15 animals each. Groups A, B and C were orally administered with 3, 6 and 9 mg/kg body weight of the CAF respectively, once daily for 14 days. Group D (control) was treated like the test groups except that the animals received distilled water (vehicle). All administrations were done daily between 08:00-08:45 h. The study was carried out following approval from the Ethical Committee on the use and care of experimental animals of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Preparation of serum and liver homogenate

After 1, 7 and 14 days of the administration, the rats were individually weighed before sacrifice. The rats were then made unconscious in a jar containing cotton wool soaked in diethyl ether. Thereafter, their jugular veins were cut; head held downwards and allowed to bleed into clean, dry centrifuge tubes. The tubes were left at room temperature for 10 min and thereafter centrifuged at 33.5 x g for 15 min. The sera were aspirated using Pasteur pipettes into clean, dry, sample bottles and were then stored at -20^{o}C for 24 h.

Immediately after the collection of blood, the rats were quickly dissected. The liver was removed, weighed again and then transferred into 0.25 M sucrose solution. The organ was later blotted with tissue paper, cut very thinly with sterile scalpel blade and homogenized in ice-cold 0.25M sucrose solution (1:5 v/v) (Akanji *et al*., 1993). The homogenates were further centrifuged at 1340 *x g* for 15 min to obtain the supernatant, which was then carefully collected into sample bottle and used for the various biochemical assays.

Determination of biochemical parameters

The functional indices of the rat liver were determined as described for ALT and AST activities (Schmidt and Schmidt, 1963), serum concentrations of total protein (Plummer, 1978), total bilirubin (Evelyn and Malloy, 1938), albumin (Doumas *et al*., 1971), globulin (Tietz, 1995) and liver-body weight ratio (Yakubu *et al*., 2003).

Histopathological examination

The method described by Krause (2001) was used for the histopathological examination. Briefly, fixed liver were dehydrated through ascending grades of ethanol to absolute alcohol (70%, 90% and 95% absolute). The liver was cleaned in xylene, impregnated and embedded in paraffin wax (melting point, 56°C), sections were cut at 5µm on a rotatory microtone. These sections were floated out on clean microscope slides which had previously been lightly albumerized (to avoid detachment from slides during staining procedure) after which they were dried for 2 h at 37°C (Drury and Wallington, 1973). The slides were observed using the Leitz, DIALUX research microscope and photomicrographs produced in bright field at a magnification of x 200.

Statistical Analysis

Data obtained were subjected to one-way Analysis of Variance (ANOVA) and means were separated by Duncan Multiple Range Test. Differences were considered statistical significant at $P < 0.05$ (Mahajan, 1997).

Results

The effect of administration of crude alkaloidal fraction from *Cnestic ferruginea* root on the liver function indices of male rats are depicted in Tables 1-10. For the acute toxicity studies, the CAF administered orally at a dose of 9 mg/kg body weight produced no death while a dose of 45 mg/kg body weight caused 100% mortality (Table 1). The CAF at doses of 18, 27 and 36 mg/kg body weight caused death in 17, 50 and 83% of the animal tested respectively. Similar to result obtained in the work of Rujjanawate *et al* (2003), observable changes in behavior after the lethal dose administration were decrease in motor activity, analgesia and decrease in respiratory rate. The LD_{50} of the CAF in male albino rats calculated from the data is 27 mg/kg body weight For the sub-acute toxicity studies, the 3 and 6 mg/kg body weight of the alkaloid fraction did not manifest any effect on the liver AST activity until the

seven daily doses when the enzyme activity was increased by 37.08 and 2.10% respectively. While the increase in the enzyme activity was sustained throughout the remaining experimental period in the 3 mg/kg body weight of the extract treated animals, those administered with 6 mg/kg body weight of the extract had their enzyme activity reduced. The highest dose investigated in the sub-acute study (9 mg/kg body weight) decreased the enzyme activity in the rat liver throughout the period of the experiment. The serum enzyme was however increased in all the alkaloidal extract treated animals (Table 3).

Table 1: Acute toxicity of the crude alkaloidal fraction (CAF) of *B Cnestis ferruginea* D.C root administered orally in male albino rats

Dose (mg/kg body weight)	Death/total	Death (%)
9	0/18	0
18	3/18	17
27	9/18	50
36	15/18	83
45	18/18	100

Table 2: Effect of administration of alkaloids fraction of *B Cnestis ferruginea* D.C root on liver AST (I/U) activity of male albino rats

	DAYS		
Treatment (mg/kg body weight)	1	7	14
Control	56.5±2.09[a]	56.90±1.98[a]	56.7±2.87[a]
3	56.6±2.98[a]	78.00±3.29[c]	70.01±2.91[f]
6	59.25±2.76[a]	68.75±2.07[d]	50.75±1.99[d]
9	43.5±1.98[b]	47.25±2.01[b]	44.5±1.79[b]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e, f] = p<0.05

Table 3: Effect of administration of alkaloids fraction of *Cnestis ferruginea* D.C root on serum AST activity (I/U) of male albino rats

Treatments (mg/kg body weight)	DAYS 1	7	14
Control	22.55±1.85[a]	22.62±1.87[a]	22.75±3.41[a]
3	33.75±1.98[b]	47.10±1.99[e]	45.25±2.71[e]
6	58.00±2.18[c]	48.50±1.29[e]	41.50±4.03[d]
9	41.25±3.18[d]	47.75±2.47[e]	46.50±1.85[e]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e,] = p<0.05

Table 4: Effect administration of alkaloids fraction of *Cnestis ferruginea* D.C root on liver ALT activity (U/I) of male albino rats

Treatments (mg/kg body weight)	DAYS 1	7	14
Control	38.00±2.16[a]	37.21±1.99[a]	38.00±4.87[a]
3	38.07±1.78[a]	42.50±2.19[d]	54.00±3.19[e]
6	18.75±1.47[b]	20.38±2.84[b]	23.75±3.84[c]
9	27.25±1.05[c]	20.75±5.01[b]	25.25±2.19[c]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e,] = p<0.05

Whereas the activity of the liver ALT was increased by the 3 mg/kg body weight from after seven daily doses, the 6 and 9 mg/ kg body weight decreased the activity of the enzyme throughout the experimental period (Table 4). This was however accomplished by increase in the serum enzyme throughout the period of administration.

While the administration of the crude alkaloidal fraction at all the doses significantly decreased the levels of total protein, albumin and globulin in the serum throughout the period of the experiment, the levels of total bilirubin were increased by the 6 and 9 mg/kg body weight. The 3 mg/kg body weight produced values that compared favourably with the control on all the days of intervention.

Whereas all the doses investigated increased the liver-body weight ratio by single administration of crude alkaloidal fraction, further administration resulted in significant decrease in the organ-body weight ratio throughout the remaining experimental period.

Histopathological examination of cross section of the rat liver revealed haemorrhagic features such as the presence of the red blood cells in the spaces within the hepatocytes. The severity however appears to be dose-dependent (Plates 1-4).

Table 5: Effect of administration of alkaloids fraction of *Cnestis ferruginea* D.C root on serum ALT activity (U/I) of liver of male albino rats

	DAYS		
Treatments (mg/kg body weight)	1	7	14
Control	18.50±1.39[a]	18.70±0.69[a]	18.65±0.69[a]
3	21.50±3.49[b]	29.75±0.69[d]	27.75±7.63[f]
6	22.50±1.14[c]	22.00±1.33[c]	30.75±2.37[d]
9	23.00±1.60[c]	36.50±2.40[e]	31.00±1.60[d]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e, f] = p<0.05

Table 6: Effect administration of alkaloids fraction of *Cnestis ferruginea* D.C root on serum total protein (g/L) of liver of male albino rats

	DAYS		
Treatments	1	7	14
Control	86.75±6.08[a]	86.00±4.69[a]	86.45±5.78[a]
3	54.25±2.67[b]	56.50±3.87[c]	45.00±2.18[d]
6	52.75±1.28[b]	48.00±2.10[d]	49.75±1.98[e]
9	52.00±1.59[b]	46.00±2.22[d]	43.00±1.93[d]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e,] = p<0.05

Table 7: Effect administration of alkaloids fraction of *Cnestis ferruginea* D.C root on serum albumin (g/L) of liver of male albino rats

Treatments (mg/kg body weight)	DAYS 1	7	14
Control	30.50±2.38[a]	30.00±4.80[a]	30.25±2.37[a]
3	21.00±1.69[b]	20.90±1.59[d]	27.75±2.08[e]
6	20.25±4.12[b]	23.75±2.09[c]	20.25±1.71[b]
9	23.75±3.27[c]	22.00±3.21[c]	18.00±3.20[b]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e,] = p<0.05

Table 8: Effect administration of alkaloids fraction of *Cnestis ferruginea* D.C root on serum total bilirubin (µmol/L) of liver of male albino rats

Treatments (mg/kg body weight)	DAYS 1	7	14
Control	1.20±0.09[a]	1.21±0.08[a]	1.20±0.15[a]
3	1.23±0.27[a]	1.35±0.18[c]	1.23±0.14[a]
6	1.50±0.10[b]	1.98±0.45[d]	1.65±0.05[f]
9	1.49±0.07[b]	1.85±0.06[e]	1.60±0.08[f]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e, f] = p<0.05

Table 9: Effect administration of alkaloids fraction of *Cnestis ferruginea* D.C root on serum globulin concentration (µmol/L) of male rat liver

Treatments (mg/kg body weight)	DAYS 1	7	14
Control	56.25±1.52[a]	56.00±1.01[a]	56.20±1.28[a]
3	33.25±1.02[b]	35.60±0.72[b]	17.25±0.63[b]
6	32.50±1.12[b]	24.25±0.51[c]	29.50±0.72[c]
9	28.25±0.30[c]	24.00±0.52[e]	25.00±0.14[c]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e, f] = p<0.05

Table 10: Effect of administration of alkaloids fraction of *Cnestis ferruginea* D.C root on liver- body weight ratio of the albino rats

Treatments (mg/kg body weight)	DAY 1	DAY 7	DAY 14
Control	0.032±0.004[a]	0.032±0.004[a]	0.032±0.005[a]
3 mg/kg body weight	0.039±0.003[b]	0.027±0.001[e]	0.029±0.001[e]
6 mg/kg body weight	0.034±0.001[c]	0.028±0.001[e]	0.022±0.004[f]
9 mg/kg body weight	0.037±0.002[d]	0.021±0.002[f]	0.025±0.001[g]

Data are expressed as mean ± S.E.M (n=5)
[a] = p> 0.05, [b, c, d, e, f, g] = p<0.05

Discussion

Herbal medicine is gaining popularity in developing countries as it has been estimated that 80% of the world population still depend mainly on traditional medicine and traditional treatment involving the use of plant extract (WHO, 2000) therefore, there is the need to provide information on their safety or adverse effect of this remedy, despite the wide spread use of this medicinal plants, few scientific studies have been undertaken to ascertain the safety of this plant the present studies.

The biochemical indices monitored in the liver and serum in this study are useful markers for accessing the functional capacities of the organ. Biochemical indices of organ function if altered will impair the normal function of the organ (Afolayan and Yakubu, 2009).

Aminotransferases ALT and AST are useful markers of liver cytolysis (Yakubu *et al*.,2005) the elevated liver AST observed with the 3mg/kg body weight can be adduced to increase in the functional activity of the organ

leading to the enzyme synthesis however, the reduction of liver AST activity observed with 6 and 9 of the crude alkaloidal fraction may be attributed to changes in membrane permeability of the cells (Jimoh and Odutuga, 2001) which in this studies was supported by increase in the serum enzyme. The alteration produced in the liver AST and ALT activity are indications of adverse effect on the liver it further suggests that the crude alkaloidal fraction of *Cnestic ferruginea* 6 and 9 may be cytotoxic to the liver suggest may adversely affect the metabolism of aminoacids in the animals this findings agrees with that of Afolayan and Yakubu (2009) following the administration of *Bulbine natalensis* stem to male rats.

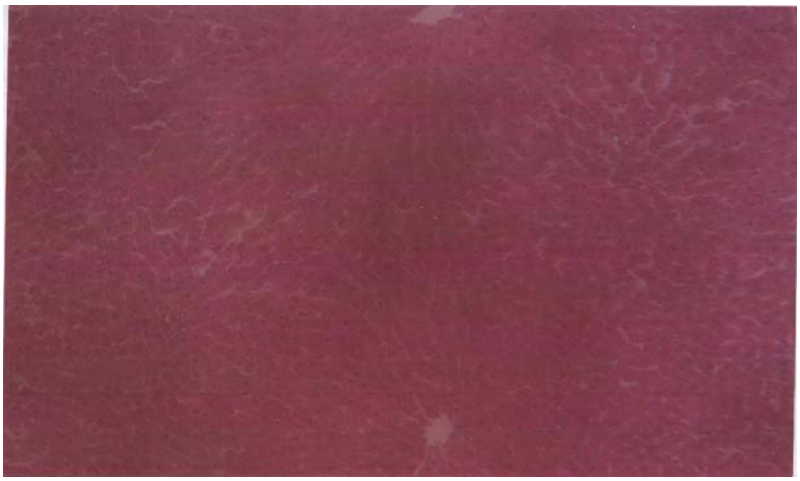

Plate 2a: Photomicrograph of hepatic plate (normal architecture) from the liver of control rats placed on distilled water for 14 days (x 200)

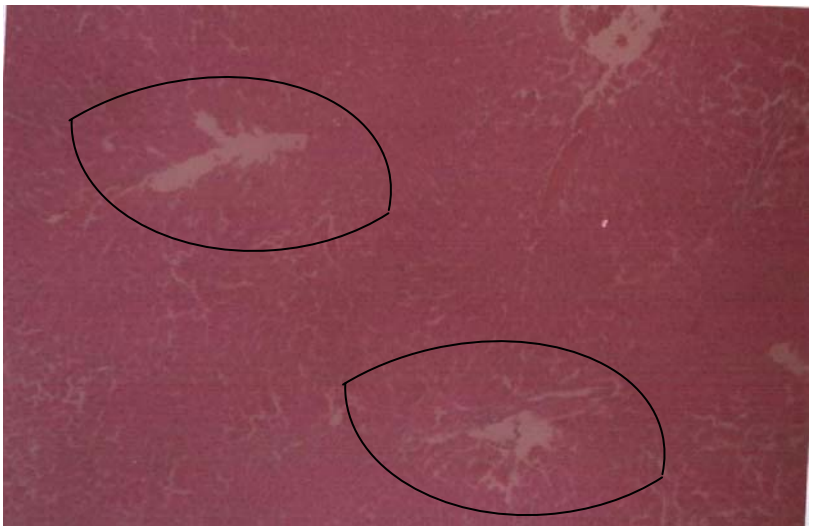

Plate 2b: Photomicrograph of hepatic plate of liver of rat administered with 3 mg/kg body weight of alkaloids fraction of *B Cnestis ferruginea* D.C root for 14 days (x 200). The circled areas show heamorrhagic features with minor disintegration of hepatic cells

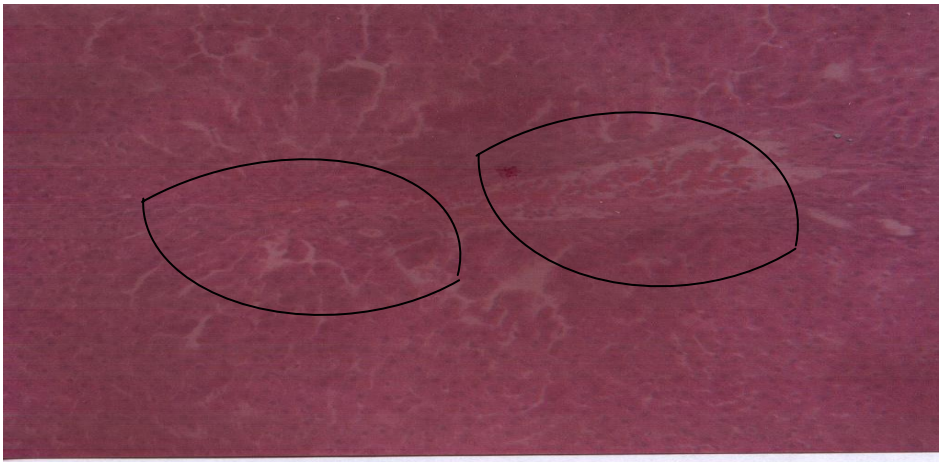

Plate 2c: Photomicrograph of hepatic plate of liver of rat administered with 6 mg/kg body weight of alkaloids fraction of *Cnestis ferruginea* D.C root for 14 days (x 200). The circled areas show heamorrhagic features with intensified disintegration of more open spaces

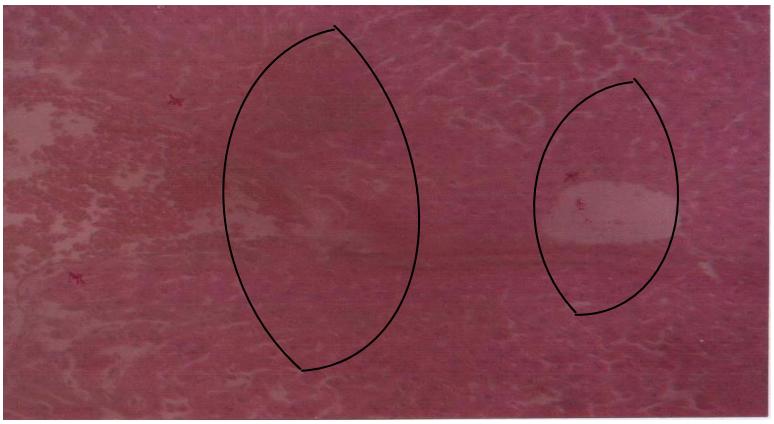

Plate 2d: Photomicrograph of hepatic plate of liver of rat administered with 9 mg/kg body weight of alkaloids fraction of *Cnestis ferruginea* D.C root for 14 days (x 200). The circled areas show heamorrhagic features with gross tissue disintegration and breakage typified by large open spaces

Albumin, total bilirubin and globulin are mixtures of protein molecules that can be used to indicate the integrity of glomeruli tubular and regulation of osmotic pressure (Guyton and Hall, 2000) in addition to the total protein as well as the mixtures of these proteins can be used to access the secretory ability of the secretory ability of the liver (Yakubu *et al*., 2003) in this study it was not all the secretory component of the liver function that were affected in the same partern in this study this might be an indication of parameter specific response by the animals to the fraction.

The reduction in total protein, albumin, and globulin are indications of diminished synthetic function of the liver which might be a consequence of impair hepatocellular function, low albumin content in the serum may also suggest liver damage, infection or may also be due to continuous loss of albumin (Tietz *et al*., 1994; Yakubu *et al*.,2003). Serum globulin, a heterogeneous complex mixture of protein molecule whose fractions are altered characteristically in different disease conditions. Incase of chronic infection a reduction in albumin is always accompany by corresponding increases in globulin however, since globulin and albumin are both reduced in this study it may therefore be logical to conclude that the extract did not cause any infection but a case of liver damage. Bilirubin is an important catabolic product of the blood and its biological and diagnostic values have been established (Tietz *et*

al., 1994; Yakubu *et al*., 2003) therefore the increase in the bilirubin content suggest adverse effect on liver function this is similar to what was obtained by Afolayan and Yakubu (2009).

The fact that the 3 mg/kg body weight of the crude alkaloidal fraction produced values of total bilirubin that compare well with the control suggests that the dose might not have accumulated to a critical level that would have manifested adverse effect on the liver. It is also possible that the animal to adapt favourably to the effect of the crude alkaloidal fraction at this dose. This also connotes dose specific effect of the crude alkaloid fraction on the liver parameters.

Organ-body weight ratio can be use to indicate organ swollen, atrophy or hypertrophy (Moore and Dalley, 1999) while the increase in liver-body weight ratio by the single administration crude alkaloidal fraction may be adduced to possible adaptation of the animals to the assault of crude alkaloidal fraction the sustained decreases in the organ-body weight ratio could possibly be due to constriction of the hepatocytes.

The dose dependent hemorrhagic features observed in this study further lend credence to the adverse effect of crude alkaloidal fraction. Our present findings have shown that crude alkaloidal fraction of *Cnestic ferruginea* root is not completely safe at the doses investigated

The alterations produced on the liver functional indices suggest adverse effect on the liver probably because it's a vulnerable target to a number of toxicants since it metabolises foreign substances. This hepatotoxic effect of the crude alkaloidal fraction will adversely affect the normal function of the liver and thus the root may not be completely safe for consumption.

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