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Hepatoprotective and antioxidant activities of the separate and combined administration of methanolic extract of *Adasonia digitata* and *Cochorous olitorious* leaves on Rats

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Abstract

The hepatoprotective and antioxidant activities of the separate and combined administration of methanolic extract of combine administration of *Adasonia digitata* and *Cochorous olitorious* leaves on Rats was investigated. During which Forty –two rats were grouped into 7 groups of 6 animals each. Group A rats received 2mL of distilled water, while groups B, C,D,E,F and G received equal volume corresponding to 500 and 1000 mg/kg body weight of the extract, respectively, for 14 days. The rats were sacrificed 24 h after 14days administration. The qualitative and quantitative phytochemical constituent of methanolic extracts of *Adasonia digitata* and *Cochorous olitorious* leaves was screened and was said to contain Saponins (*A.dgitata:*16.58 and *C.olitorous*:22.17), alkaloids (*A.dgitata:*81.56and *C.olitorous:*68.65), tannins (*A.dgitata:*311.98and *C.olitorous:*287.07*)*, phenolics (*A.dgitata:*170.90and *C. olitorous:* 330.07), flavonoids (*A.dgitata:*25.38and *C.olitorous:*157.38). The level of liver serum alanine amino transferases (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) shows no significant ($P<0.05$) differences from the control group. Similar result was also obtained from the liver histological profile showing no visible damage when compared with the control group. There was no significance ($P<0.05$) differences between the heamatological profile, protein level, total bilirubin in the administered group when compared with the control group. The level of Superoxide dismutase, Catalase, Glutathione-S-transferase, Glutathione peroxidase enzymes were significantly ($P<0.05$) increase in the administered group when compared with the control group. It is therefore logical to conclude that, the plant may be explored as oral remedy in the treatments of free radical induced and inducing diseases.

Keywords: *Adasonia digitata* ,*Cochorous olitorious*, Histopathology, Antioxidant and Hepatoprotective

1. Introduction

Herbal medicine is gaining popularity in developing countries as it has been estimated that 80% of the world population depend mainly on traditional medicine involving the use of plant extract (WHO, 1999). Various plants have been reported to be used in the treatment of several ailments.

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These include *Pterocarpus osun* used in the treatment of eczenma, acnes, asthma and candidiasis (Gill, 1992), as well as *Annona senegalensis*in the treatment of diarrhoea, disease of the joints, respiratory diseases, conjunctivitis, wounds, snakebites, trypanosomiasis, jaundice, haemorrhoids, feminine barrenness, convulsions, ovarian cancer, fever, and asthenia (Neuwinger, 1996). *Occimum grattisum*has also been established to be antianeamic, anticancer and anti-diarrhea (Neuwinger, 1999).*Adasonia digitata and Cochorous olitorious* have been claimed to be used in folklore medicine without recourse to the safety and efficacy of the combine administration on enzyme system and membrane integrity

Adansonia digitata

Commonly used traditional plants, consumed in food or used in the direct treatment of several diseases such as cancer, anaemia, diabetes, ischemia reperfusion diseases and inflammatory bowel syndrome in South-western Nigeria. *A.digitata* is a tree found widely throughout Africa and known locally in African countries as tree of life due to its ability to sustain life, as well as its many medicinal and nutritional uses (Lewanda et al., 2007). *A. digitata*is commonly called tree of life or Baobabs in English; Kukah in Northern Nigeria, 'Luru' in South-Western Nigeria, Konian in Southern Nigeria. The tree is ubiquitously found in the tropics and Savanah forest.

The leaves are used medicinally as a diaphoretic, an astringent, an expectorant and as prophylactic against fever (Bryo *et al*., 2007). Baobab leaves have been investigated and found to contain bioactive secondary metabolite that may be responsible for the treatment of certain ailments, as well as containing properties that can be beneficial to overall health. Examples of such bioactive compounds includes tannins which has been established to prevent abortion by directly preventing anti-nutrient activities against the uterine wall of female fallopian tubes (Yakubu *et al*., 2007), Saponins which prevent blood damage by maintain the discocytic structure of heamoglobin and acting as blood tonic and purifiers (Oladiji *et al., 20*07), flavonoids and polyphenol present in *A. digitata* have been established to induced Heam Oxygenase (HO-1) , which have been implicated in heme degradation (Farombi *et al*., 2001).

Corchorus olitorous

According to a reviewed work by Hamzah *et al*., 2001, *Corchorus olitorius (*Linn) is aleafy vegetable that belongs to the family *Tiliaceae*, and commonly called Jute mallow in English and "Ewedu" in the South-western Nigeria. It is an animal herb with a slender stem and an important green leafy vegetable in many tropical area including Egypt, Sudan, India, Bangladesh. The leaves (either fresh or dried) are cooked into a thick viscous soup or added to stew or soup and are rich sources of vitamins and minerals (Branda *et al*., 2004). Nutritionally, *C. olitorius* on an average contain 85-87g H_2O, 0.7g oil, 5g carbohydrate, 1.5g fiber, 250- 266mg Ca, 4.8mg Fe, 1.5mg 300010 vitamin A, 0.1mg thiamine, 0.3mg riboflavin, 1.5mg nicotinamide, and 53-100mg ascorbic acid per 100g (Branda, 2004).

The leaf extract of the plant is also employed in folklore medicine in the treatment of gonorrhea, pain, fever and tumor (Branda *et al*., 2007). It is reportedly consumed as healthy, vegetable in Japan because of its rich contents of carotenoids, vitamin B1, B2, C and E, and minerals (Chihande *et al*., 1997). Its leaves and roots are eaten as herbal medicine in South-East Asia. In some part of Nigeria, the leaves' decoction is used for treating iron deficiency, folic acid deficiency, as well as anemia, it's also act as blood purifier and the leaf twigs is used against heart troubles (Krivanek *et al*., 2007) while cold leaf infusion is taken to restore appetite and strength. The leaves are used for ascites, pains, piles, tumors, gonorrhea and fever (Dharmendra *et al*., 2006).

The administration of the individual plant has been established to be antipyretic, a rubefacient, antianeamic, antidiabetic, anti-inflammation etc., and to counter irritation as well as to manage conditions such as bladder, kidney, skin and urinary diseases. Others include treatments of rheumatoid arthritis, snake and insect bites (Quisumbing, 1951; Irvine, 1961; Nadkarni, 1976; Gill, 1992; Ashton *et al*., 1997; Gupta *et al*., 2006; Maruthupandian and Mohan, 2010; Ogbole *et al*., 2010). There are several scientific reports on the constituents and pharmacological activities of the plant. For instance, the stem bark has been reported to contain phenols, saponins, flavonoids, tannins, terpenoids, alkaloids and cardiac glycosides (Patil and Gaikwad, 2011).

Furthermore, the inhibition of the growth of some pathogenic fungi and bacteria species, anti-mutagenic activity of the leaves and immune suppressing activity of the terpenoid (lupeol) isolated from the plant have been scientifically validated (Bani *et al*., 2006; Sahoo *et al*.,

2008; Chichioco-Hernandez and Paguinan; 2009; Patil and Gaikwad, 2011). Despite these pharmacological reports and wide spread use of the plant, information on the safety or toxicity appears scanty. Therefore, this study was set out to assess the safety of the methanolic extract of the combined administration of *A. digitata* and *C. olitorious* on the heamatological, biochemical, lipid profile, antioxidant parameters and maintenance of organ integrity. The organ (liver) has been carefully selected because of its roles in the detoxification, metabolism and distribution of the plant extract. The histological examination was also carried out to corroborate it with the result obtained from the biochemical parameters. The dose of 500 mg/kg body weight was arrived at from ethno-botanical survey, while the 1000mg/kg body weight represent two-fold of the 500 mg/kg body weight, respectively and the same dose was arrived for the combine administration respectively.

The choice of the combination was stemmed to the ethnobotanical use in the treatment of epilepsy and diabetes by the herbs sellers in 'Oja tuntun' Market, Ilorin, Kwara State Nigeria, coupled with the fact that after the phytochemical screening of the individual plant it was realized that *A.digitata* have lower quantity of flavonoids and polyphenols which is compensated for by *C.olitorous.* Also *C. olitorous* poses lower tannins and Alkaloids which can be compensated for by *A.digitata.*

2. Materials and Methods

The Plant materials and authentication of the fresh leaves of *Adasonia digitata and Cochorous olitorious were* purchased from Bodija Market, Ibadan, Oyo State, Nigeria and were identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria, with the voucher number UILH/001/951/ and UILH /002/154 respectively.

Experimental Animals

Forty-two albino rats (*Rattus norvegicus*) with average weight of 200± 2.00 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ibadan, Ibadan. The animals were kept in clean plastic cages placed in a well-ventilated house with optimum condition (temperature: 22±3^{o}C; photoperiod: 12h natural light and 12 h dark; humidity: 40-45%). They were allowed unrestricted access to commercial pelletized rat chow (Ladokun Feeds and Flour Mills, Mokola, Oyo State, Nigeria) and water. The cages were cleaned daily. The study was carried out according to the Guide for the Care and the Use of

Laboratory Animals of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, USA (ILAS, 1997).

Assay kits

The assay kits for Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), bilirubin and lipid profile, were products of Randox Laboratories Ltd, United Kingdom. All other reagents used were of analytical grade and were prepared with distilled water.

Preparation of extract

The procedure described by Yakubu *et al*. (2005) was adopted for the preparation of aqueous extract of *A. digitata and C. olitorious* leaves. Briefly: the fresh leaves were oven dried at room temperature to a constant weight, pulverized into powdered form and stored in a plastic container. A known weight (500 g) of the powder was extracted in 3L of distilled methanol and then left undisturbed for 72 h after an initial vigorous stirring. This was later filtered with Whatman No.1 filter paper and the filtrate concentrated using rotary evaporator to give greenish brown slurry of 96.72g (percentage yield of 19.34%). This was then reconstituted in distilled water to give the required doses of 500 and 1000 mg/kg body weight of the extract used in the present study.

Phytochemical screening

The aqueous extract of *Adasonia digitata and Cochorous olitorious* leaves were screened for phytochemical constituents according to the procedures described by Odebiyi and Sofowora (1978). The detected phytochemical were quantified as described for alkaloids (Henry, 1973), saponin (Brunner, 1984), flavonoids (El-Olemy *et al*., 1994), phenolics (Edeoga *et al*., 2005) and tannins (Van-Burden and Robinson, 1981).

Animal grouping and extract administration

Forty-two albino rats of Wistar strain were randomly assigned into 7groups (A-G) of Six animals each. Animals in group A (control) received 2 mL of distilled water once daily for fourteen days while those in groups B and C received 500 and 1000 mg/kg body weight of *Adasonia digitata*: Groups D and E were administered 500 and 1000 mg/kg body weight of *Cochorous olitorious* and group F and G were administered the combined plant extracts in a 50:50% concentration of the two plant respectively. The animals were then sacrificed 24

hours after 14 days of administration. The summary of administration groupings of the animals is as shown in table below.

TABLE 1: Animal Groupings and Extract Administration

Groups	Treatment
Control group	Feed with distilled water + pelletized chow
E_1 500 mg/kg	Administered with 500 mg/kg *A. digitata*
E_1 1000 mg/kg	Administered with 1000 mg/kg *A. digitata*
E_2 500 mg/kg	Administered with 500 mg/kg *C. olitorious*
E_2 1000 mg/kg	Administered with 1000 mg/kg *C. olitorious*
E_1+E_2 500 mg/kg	Administered with 500 mg/kg *A. digitata* and *C. olitorious*
E_1+E_2 1000 mg/kg	Administered with 1000 mg/kg *A. digitata* and *C. olitorious*

E_1 = *A. digitata* and E_2 = *C. olitorious*

Preparation of tissue homogenates, Serum and Histology

The procedure described by Yakubu and Akanji (2011) was adopted for the preparation of serum and tissue homogenates. Briefly: The animals were sacrificed under ether anaesthesia and an aliquot of the blood was collected into a sample bottles containing EDTA for hematological analyses. Another 5mL of the blood was allowed to clot at room temperature for forty-five minutes and then centrifuged at 4500 x g for 10 min. The serum was then kept frozen for 12 h before being used for the biochemical analyses. The animals were thereafter quickly dissected and the liver removed, cleaned, weighed and stored in ice-cold 0.1 M phosphate buffer solution of pH 7.4. The organ was then homogenized in ice-cold phosphate buffer solution (1:4w/v).The homogenate was centrifuged at 10,000 x g for 15 min and the resulting supernatant stored frozen for 24 h. Some part of the tissue (liver) was cut and weighed into a universal test tube containing formalin solution to preserve the tissue for histological analysis.

Heamatological Studies

Automated Heamatological Analyzer (Sysmex Heamatology Systems, Sysmex Ameria Inc., model no. KX-21N, Kobe, Japan) was used to determine the levels of heamoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean

corpuscular heamoglobin (MCH), mean corpuscular heamoglobin concentration (MCHC), white blood cells (WBC), neutrophils, lymphocytes and platelets.

Determination of Biochemical Parameters

The biochemical analysis were determined for protein (Gornall *et al*., 1949), AST and ALT (Reitman and Frankel, 1957), ALP (GSCC, 1970), Malondealdehyde (MDA) (Doumas *et al*., 1971), Glutathione-s-transferase (GST) and Glutathione peroxidase (GPx) (Evelyn and Malloy, 1938), Catalase (CAT), Super oxide dismutase (SOD)(Tietz, 1995).

Statistical analysis

Data obtained were expressed as Mean ± Standard Deviation and analyzed using the Analysis of Variance 'ANOVA, F-ratio and student's t' test where applicable. Values at P=0.05 and P<0.05 were regarded as significant in comparison with appropriate controls.

3. Results and Discussion

Table 1: Qualitative Phytochemical Constituents of *A. digitata* and *C. olitorius*

Metabolites	***A. digitata***	***C. olitorius***
Alkaloids	++	++
Anthraquinone	-	-
Cardiac glycosides	-	-
Flavonoids	++	++
Polyphenols	++	+++
Saponin	++	++
Tannins	+++	++
Terpenoids	+	++
Steriods	+	+

+: Faintly present, ++: Moderately present, +++: Excessively present and -: Absent

Table 2: Quantitative Phytochemical Constituents of *A. digitata* and *C. olitorius*

Metabolites	*A. digitata*	*C. olitorius*
Alkaloids	**81.56 ± 0.56**	**68.65 ± 2.05**
Saponin	**16.59 ±1.85**	**22.17 ± 0.24**
Tannins	**311.98 ± 0.01**	**287.07 ±0.16**
Flavonoids	**25.38± 2.88**	**157.38 ±0.38**
Polyphenols	**170.90 ± 0.68**	**330.07 ±0.32**

The extracts were very rich in polyphenols, tannins, alkaloids and flavonoids, with Saponins inclusive as shown in table 2 above.

Table 3: Effects of administration of methanolic extracts of *A. digitata* and *C. olitorous* leaves plant on the heamatological profile of rats

Treatment Group	PCV(%)	HB(%)	RBC(%)	WBC(10^9/L)	LYMPHS	PLTSx10^5/uL
Normal Control	36.49±0.36^a	12.04±0.21^b	8.84±0.06^c	8.85±0.04^d	57.23±0.39^e	134.00±3.00^f
$E_1$500	37.03±0.89^a	11.99±0.10^b	8.85±0.38^c	9.09±0.33^d	56.33±0.33^e	133.33±1.00^f
$E_1$1000	36.26±0.37^a	11.90±0.31^b	8.86±0.24^c	9.00±0.65^d	56.13±1.15^e	132.00±8.00^f
E_2 500	35.70±0.53^a	11.91±0.20^b	8.87±0.42^c	9.10±0.57^d	56.10±1.90^e	132.00±8.00^f
$E_2$1000	36.33±0.38^a	11.98±0.36^b	8.86±0.15^c	9.23±0.16^d	57.00±1.15^e	131.40±1.00^f
E_1+$E_2$500	36.60±0.70^a	12.04±0.31^b	8.87±0.03^c	9.00±0.71^d	56.73±1.15^e	132.67±1.00^f
E_1+$E_2$1000	37.02±0.40^a	12.00±0.10^b	8.83±0.27^c	9.09±1.00^d	56.00±1.16^e	133.33±8.00^f

Values are expressed as Means ± SEM; n=7, E_1: *A. digitata*. E_2: *C. olitorous*. Significant difference of $p<0.05$. After 14 days of administration period. Heamoglobin (Hb), Park Cell Volume (PCV), White Blood Cell Count (WBC), Mean Corpuscular Volume (MCV), Red Blood Cell Count (RBC), Platelets and Lymphocytes of the animals throughout the experimental periods.

Table 4: Effects of administration of methanolic extracts of *A. digitata* and *C. olitorous* leaves plant on the Endogenous Antioxidants Status of rats

Treatment Group (Umol/mg protein)	GST	GPX	CAT	SOD	MDA
Normal Control	25.70 ± 0.41^a	2.67 ± 0.01^a	57.88 ± 0.01^a	4.61 ± 0.01^a	0.230±0.03^a
$E_1$500	29.17 ± 0.24^b	2.80 ± 0.01^b	59.10±0.11^b	5.04 ±0.07^b	0.245±0.01^b
$E_1$1000	33.50 ± 0.18^c	3.09 ± 0.01^c	64.40 ± 0.30^c	5.56 ± 0.08^c	0.240±0.04^c
E_2 500	30.71 ± 0.36^b	2.90 ± 0.02^c	60.00 ± 0.22^b	5.004 ±0.02^b	0.242±0.01^d
$E_2$1000	34.92 ± 0.08^c	3.12 ± 0.01^c	65.25 ± 0.27^c	5.60 ± 0.01^c	0.246±0.03^c
E_1+$E_2$500	38.40 ± 0.03^d	3.44 ± 0.01^d	69.45± 0.29^d	6.10 ± 0.11^d	0.230±0.05^d
E_1+$E_2$1000	41.52 ± 0.05^e	3.71 ± 0.01^e	73.78± 0.40^e	6.14 ± 0.32^e	0.224±0.06^a

Values are expressed as Means ± SEM; n=7, E_2: *C. olitorous*; E_1: *A. digitata*. Significant difference of $p<0.05$. The extract significantly increases ($P<0.05$) the induction of the liver SOD, CAT, GST, GPx and GSH status except for MDA that shows no significant($P<0.05$) differences when compared to normal control group at the varying doses as shown in table 4.

Table 5: Effects administration of methanolic extracts of *A. digitata* and *C. olitorous* leaves plant on the activities of serum AST, ALT, ALP of rats for 14 days.

Treatment Group	AST(U/l)	ALT(U/l)	ALP(U/l)
Normal Control	95.39 ± 0.89[a]	58.23 ± 0.51[a]	24.68 ± 0.16 [a]
$E_1$500	94.69 ± 0.19[a]	57.67 ± 0.33 [a]	24.33 ± 1.20 [a]
$E_1$1000	94.78 ± 0.19[a]	56.80 ± 0.58 [a]	24.00 ± 0.58 [a]
E_2 500	95.00 ± 0.58[a]	57.67 ± 0.33 [a]	25.82 ± 0.74 [a]
$E_2$1000	96.07 ± 0.89[a]	58.07 ± 0.44 [a]	25.00 ± 0.58 [a]
E_1+$E_2$500	94.61 ± 1.73[a]	58.80 ± 0.18 [a]	25.00 ± 0.21 [a]
E_1+$E_2$1000	95.97 ± 1.20[a]	58.02 ± 0.29 [a]	25.33 ± 0.33 [a]

Values are expressed as Means ± SEM; n=7, NR; E_2: *C. olitorous*; E_1: *A. digitata*. Significant differences of $p<0.05$. By the end of the 14 days period of administration, the activities of the hepatic enzymes (ALT, ALP, AST) is not significantly ($P=0.05$) different from the control group at all the varying doses of the plant extract as shown in table 5.

Table 6: Effects of 14days administration of methanolic extracts of *A. digitata* and *C. olitorous* leaves plant on serum level of Total Protein (TP) and bilirubin (TB) in Wister rats

Treatment Group	TP(umol/mg protein)	TB (umol/mg protein)
Normal Control	92.17 ± 0.89[a]	14.07 ± 0.19[a]
$E_1$500	91.20 ± *0.46*[a]	13.76 ± 0.41[a]
$E_1$1000	91.97 ± *0.23*[a]	13.79 ± 0.16[a]
$E_2$500	90.70 ± 0.30[a]	13.14 ± 0.60[a]
E_2 1000	92.14 ±0.10[a]	13.91 ± 0.31[a]
E_1+$E_2$500	91.85 ± 0.20[a]	13.99 ± 0.10[a]
E_1+$E_2$1000	91.63 ± 0.80[a]	13.42 ± 0.12[a]

Values are Expressed as Means ± SEM; n=7, E_1: *A. digitata* E_2: *C. olitorous*; Significant difference of $p<0.05$. The total protein and total bilirubin of the administered group was maintained throughout the 14 days periods of administration and there were no significant ($P<0.05$) differences between the administered and control group as shown in table 6 above.

Table 7: Effects of methanolic extracts of *A. digitata* and *C. Olitorous* on average body weight of experimental rats per group

Treatment Group	Initial bwt. (gm)	Final bwt. (gm)	Increase in bwt. (gm)
Normal Control	199.42 ± 0.59^a	209.00 ± 0.33^a	8.58±0.26
$E_1$500	200.33 ± 0.33^a	217.67 ± 0.58^b	17.34±0.25
$E_1$1000	201.67 ± 0.33^a	223.33 ± 0.58^c	21.66±0.25
E_2 500	201.00 ± 0.58^a	217.33 ± 0.58^b	16.33±0.00
E_2 1000	197.67 ± 0.33^a	223.33 ± 3.05^c	25.66±2.72
E_1+$E_2$500	190.00 ± 0.58^a	227.00 ± 1.00^d	37.00±0.42
E_1+$E_2$1000	197.67 ± 0.33^a	229.33 ± 0.58^e	31.66± 0.25

Values are expressed as means ± SEM; n=7, bwt: Body weight. E_1:*A. digitata*,E_2: *C. olitorous*; Significant difference of $p<0.05$. gram (gm)There was significant ($P<0.05$)increase in the body weight of the experimental rat administered with the various doses of extracts of the plant extract when compared to the control group as shown in table 7 above.

Histological Profile

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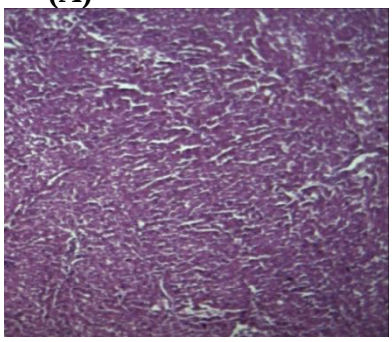
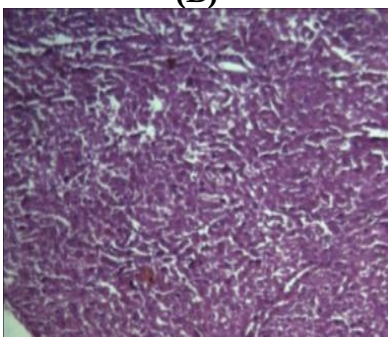
(A)



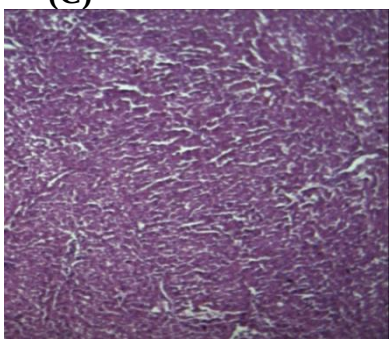
Control Group

(B)



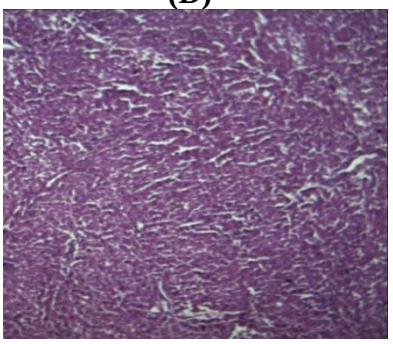
$E_1$500 mg/kg bwt .

(C)



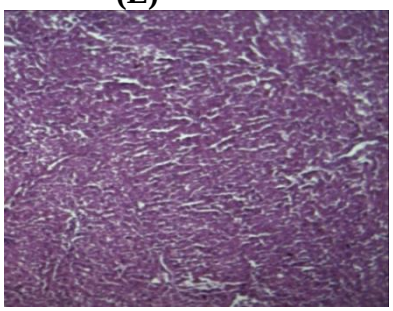
$E_1$1000 mg/kg bwt

(D)



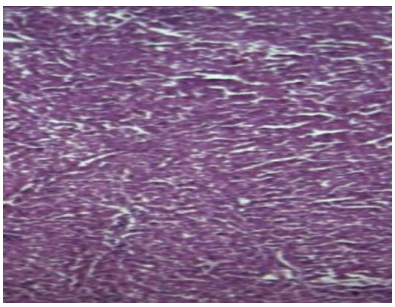
$E_2$500 mg/kg bwt.

(E)



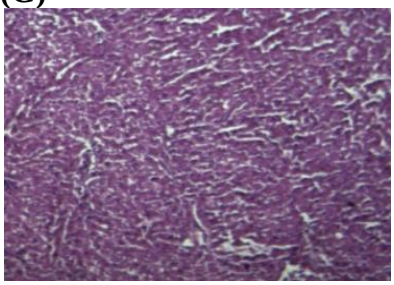
$E_2$1000 mg/kg bwt.

(F)



E_1+$E_2$500mg/kg bwt

(G)



E_1+E_2 1000mg/kg bwt

Figure 1: Liver histopathology of male Wister rats administered on methanolic extract of *A.digitata* and *C. olitorous*. E_1: *A. digitata* and E_2: *C. olitorous* at 500 and 1000mg/kg body weight. There was no macular degeneration or Periportal formation, suggestive of no damage to the hepatocytes**.** The extract at the varying dose of 500 and 1000 mg/kg body weight maintained the integrity of the histo-architecture of the liver of the animals as shown in Figures 1. above. The histo-architecture of the liver was well preserved which is devoid of any mild or macular, lesion and degeneration and congestion of the hepatocytes. Magnification of x 400.

The presence of various secondary metabolites would confer different chemical properties on plants, as well to influence their biological and toxicological effects. For example, tannins, which have been reported along with flavonoids and phenolics to have antioxidant activities, tannins may also have negative effects by chelating iron and thus impairing its bioavailability, suggestive of anaemia if individually consumed for a prolong period (Yakubu *et al*., 2007). Saponins have been reported to prevent colon cancer, possess anti-hyperglycaemic potentials and act as a tonic in aneamic patient as well as preventing blood aggregation and oligomerization of heamoglobin (Malinow *et al*., 1977; Rao and Sung, 1995; Olaleye, 2007). On the other hand, they have also been reported to have a wide range of effects such as life-threatening toxicity for certain animal species (Shidelar, 1980), disruption of biological membranes resulting in escape of large quantities of metabolites and generation of free radicals (Francis *et al*., 2002; Nandi *et al*., 2004; Sparge *et al*., 2004). However the documented toxicity of tannins, saponins and others, is due their excessive presence in the plant extract at a very high concentration when compared to other metabolite that could prevent or attenuate their anti-nutrient and iron chelating activities.

Such metabolites that could prevent the toxicity effect of tannins and saponins are flavonoids, polyphenols and alkaloids etc. When they are present in equimolar concentration or even more than the anti-nutrient metabolites, the toxicity can be attenuated, such is what was established in this study. Therefore, the documented hepatoprotective, antioxidant inducing activities and the toxicity profile of the methanolic extract of *A. digitata and C.*

*olitorous*leaves reported in this study may be attributed to separate or combination of the phytochemicals in the botanical.

Heamatological analysis provides a valuable tool that can be used to clinically investigate and assess the health status of an animal, as it plays an important role in the physiological, nutritional and pathological status of an animal (Kakade *et al*., 1972; Babatunde *et al*., 1992). It can be used to determine the toxicity effects caused by invasion of foreign substances (antigens or xenobiotics) in the blood and also explain the blood-related functions of chemical compounds including those of plant origin (Yakubu *et al*., 2007). The lack of effect on RBC and other parameters relating to it (RBC, Hb, PCV, MCH, MCHC, and MCV) as well as some relating to WBC such as platelets, lymphocytes and neutrophils suggest that the rate of synthesis or destruction of these blood cells was not adversely affected and further emphasizes the non-toxicity of the extract. White blood elicits antibodies against foreign invaders, pathogens or xenobiotics.

Elevated values may indicate a boost in immunological activity, a pathological condition, infection, severe physical stress which may upshot as a result of toxicity (Qiao *et al*., 1991; Dean, 2005; Singh *et al*., 2008). Therefore, the slight initial shoot-up levels of WBC might be an indication that the extract was initially seen foreign but was restored to after sometimes, indicating that the rate of synthesis of the blood indices over the rate of destruction or clearance from the biological fluid was control by the plant extract (Yakubu and Musa, 2012).

Farombi and Yong Jhur Shur (2001) established that, the presence of polyphenol in Cucumin is responsible for the induction of several phase-2 and antioxidant enzymes (glutathion peroxidases, heam oxygenases, superoxide dismutase, catalase, lactoferine, tranferine, ceruloplasmin, albumin etc). The presence of an appreciable amount of polyphenol in the extracts of *C. olitorius and A. digitata* maybe suggestive of the extracts ability to induce several of this enzymes, which have been implicated in free radicals scavenging potentials. The presence of tannins, saponin, polyphenols, flavonoids and alkaloids as revealed in the results of phytochemical analysis of the methanolic extract of *C. olitorius* and *A. digitata* suggests it use in folk medicine. Alkaloids are the most efficient therapeutic plant substance as an anticancer agent (Branda *et al*., 2004). Both natural and synthetic alkaloids are used as

basic medicinal agent because of their analgesic, antispasmodic and antibacterial properties (Yakubu *et al*., 2011).

Hippocrate 2000 years ago, said, let thy food be thy medicine and medicine be thy food. The administration of the extract of *A.digitata* and *C.olitorous*, may be responsible for the increase feeding level of the animal and maybe in part responsible for their increase weight as established from the study. We may therefore infer that the extract increases the level of ghrelin secretion. Ghrelin stimulate appetite for food. Flavones a member of the polyphenol family was established to mediate ghrelin secretion from the brain, so also is fructose (Adaramoye, 2011). The presence of quantifiable amount of polyphenol in the extract of *A.digitata* and *C.olitorous*may therefore be responsible for the increase appetite for food by the experimental animal in this study an invariable responsible for the weight increase.

ALP , ALT and AST are markers of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Pramyothin *et al*., 2006). The normo-regulation in the activity of the aminotransferases in the liver without a corresponding increase in the serum could be due to the ability of the extract to protect the integrity of the hepatocyte (Akanji *et al*., 1993), this is because the loss or induction in the activity or of these enzymes in the liver will adversely affect carbohydrate and amino acid metabolism, thereby affecting energy production. It appears that the extract was able to normalized and keep the activities of aminotransferases of the administered group to that which favorably compare with the control group.

The concentrations of total protein and bilirubin are useful ‘markers’ of secretory, synthetic and excretory functioning of the liver (Yakubu and Musa, 2012). The unaltered concentration of total protein in the study may suggest that there was no compromise of the synthetic ability of the liver. This was further supported by a similar normo-concnetration of bilirubin content of the animals. The extract did not increase or reduced the metabolic functional activity of the liver by interfering with the metabolism of total protein and bilirubin while it might not have interfered with the equilibrium in the rate of synthesis and destruction, removal or clearance of bilirubin from the system of the animals. Increase or decrease in total protein from normal could, however, lead to dehydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals.

The concentration of bilirubin is a biomarker to determined heam degradation by heam oxygenases-1. Heam is a component of heamoglobin, an iron prosthetic group, the higher the catabolism of heamoglobin, the more available heam for degradation by heam oxygenases-1 (HO-1) to produce bilirubin (Krivanek *et al*., 2007). So the extract did not interfere with heamoglobin lysis (as shown by the heamatological analysis), which prevent the heam from not been available for degradation to bilirubin by HO-1.

Therefore, the absence of macular degeneration and periportal formation of the hepatocytes surrounding the central vein of the liver investigated in this study, despite the proximity and the involvement of the organ in detoxifying foreign compounds, including plant extracts is indicative of the hepatosafety of the plant extract. The ability of the liver to function without compromising integrity after the administration of the extract suggests no structural or function toxicity or chronicity of the plant extract.

4. Conclusion

In conclusion, the results of this study shows that, the administration of aqueous extract of *A. digitata* and *C.olitorous* plants at the doses of 500 and 1000 mg/kg body weight caused no selective or reversible changes in the heamatological profile, body weight, protein and bilirubin level and biochemical parameters in the liver of the animals except for the antioxidant system status that was appreciably induce and boosted in the administered group. The extract also exhibited no structural and selective toxicity on the histo-architecture of liver of the animal. It is therefore logical to recommend the plant for a prophylactics and preventive measure against series of free radical induced disorders owing to it antioxidant inducing potential.

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