Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(1): 160-166
Received: 22-11-2016
Accepted: 23-12-2016

Joseph Anna UBON
Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Akwa Ibom State, Uyo, Akwa Ibom State, Nigeria

Monday Isaiah Akpanabiatu
Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Akwa Ibom State, Uyo, Akwa Ibom State, Nigeria

Edet Okon Akpanyung
Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Akwa Ibom State, Uyo, Akwa Ibom State, Nigeria

Usenobong Friday UFOT
Department of Biological Sciences, Faculty of Natural and Applied sciences, Akwa Ibom State University, Ikot Akpaden, Mkpat Enin L.G.A., Uyo, Akwa Ibom State, Nigeria

Correspondence
Joseph Anna UBON
Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Akwa Ibom State, Uyo, Akwa Ibom State, Nigeria

Effects of ethanolic extracts of *Cola millenii* K. Schum seed on biochemical and toxicological indices of male wistar albino rats

Joseph Anna UBON, Monday Isaiah Akpanabiatu, Edet Okon Akpanyung and Usenobong Friday UFOT

Abstract
Cola millenii is a medicinal plant believed to have many agents acting in different ways for the health benefits of humans. This study investigated the acute toxicity, phytochemicals content and the effects on haematological indices, lipid profile, and biomarkers of cardiac and prostate dysfunction. Twenty eight (28) adult male Wistar albino rats weighing 151-195 g were used in four groups of six animals. Group 1 (control) received 0.34 ml of 20% Tween® 80 as placebo, groups 2, 3 and 4, received intraperitoneal injection of 150, 300 and 450 mg/kg body weight of the extract respectively for seven days. The result indicates the presence of alkaloids, terpenes, saponins and cardiac glycosides. Intraperitoneal LD_{50} was 2645.75 mg/kg body weight. There was significant (p<0.05) dose dependent decrease in CK, CK-MB, LDH-L, AST, ALT and ALP activity of the test groups compared to the control. Equally, PSA, TC, TG and LDL-C decreased significantly compared to the control. Haemoglobin, PCV, and MCV also decreased significantly while HDL-C, testosterone level, platelets count, and GGT activity increased significantly. There were no significant changes in serum 5-alpha reductase as well as RBC, MCH, MCHC, lymphocytes and neutrophils counts. The results suggest that the extract may possess non-cytotoxic, cardio-protective and hepato-protective properties and may be useful in the management of BPH. However, the extract should be used with moderation as it is capable of causing reduction in Hb and PCV.

Keywords: *Cola millenii*, PSA, testosterone, 5-alpha reductase, lipid profile, haematological indices

1. Introduction
There is an increasing focus on the use of medicinal plants and their bioactive agents in drug design and development in recent decades. Medicinal plants are those plants with recognized medical applications as a result of inherent phytochemicals that are capable of acting alone or synergistically to prevent, cure or control pathological states (Okigbo, Eme and Ogbogu, 2008; Soetan and Aiyelaagbe, 2009) [39, 46]. They are important source of new chemical substances with potential therapeutic effects (Farnsworth, 1984; Akpanabiatu *et al.,* 2006; Edem, 2009a; Akpanabiatu *et al.,* 2012) [2, 3, 5, 36]. From time immemorial, Medicinal plants and their derivatives have been used in the prevention and/or management of diseases such as cancer, convulsion, skin infections, hypertension, diabetes, sexually transmitted infections and cardiovascular diseases. Consequently, medicinal plants, bioactive agents, and their derivatives offer opportunities for disease management, especially where current orthodox treatment methods have often failed (Denmeade and Isaacs, 2004; Wang *et al.,* 2006; Richter *et al.,* 2007; Pucar *et al.,* 2008) [13, 51, 42, 41]. Reliance on the use of medicinal plants has been traced to their traditional uses in folk medicine as well as the extraction and development of several drugs and chemotherapeutics from them (WHO, 1977; UNESCO, 1998) [52, 50]. Some selected examples include, benzylisoquinoline alkaloid (papaverine), antileukaemic alkaloids, vinblastine and vincristine (Nelson, 1982; Ifere *et al.,* 2009) [35, 24], the isoquinoline alkaloid, (Itharat and Ooraikul, 2007) [25], quinine (Obasi *et al.,* 2010) [37], vinca alkaloids, camptothecin, terpene paclitaxel, artesunate and the lignin podophyllotoxin (Bolk, 2001; Efferth, 2006) [9, 16]. Hence, phytochemicals are vital to human health and play significant roles in disease prevention, management and control. As research tools advances, more bioactive agents are likely to be discovered both in new medicinal plants and in those that have been investigated and their bioactive agents reported.
This knowledge has stimulated renewed interest in the possible application of phytochemicals in the development of novel drugs for human disease management considering the increasing cost and side effects of synthetic drugs.

Numerous reports on the pharmacology of herbal medicines from almost all parts of the world are scattered in various literature (Akpanabiatu *et al.,* 2009a, 2009b, 2012; Bisong *et al.,* 2012; Nwankpa *et al.,* 2012) [3-5, 36, 8, 36]. Research contributions are also available on the chemical composition of some lesser-known tropical plants that may have long been used in traditional medicine (Essien *et al.,* 1995; Demo *et al.,* 2005) [17, 12]. The search for new therapeutic agents of plant origin in disease management is therefore a global concern. There is therefore the need to explore plant materials for pharmacological and biochemical activities, with the aim of developing new drugs (Lucy and Edgar, 1999) [32]. Such exploration and bioprospecting requires concerted effort and commitment of the academia, pharmaceutical companies, research institutes and other stakeholders (Latha and Kannabiran, 2006) [29].

Cola millenii K. Schum is a small tree 3-6 m high, with a low crown of arching branches and edible fruits found in deciduous, closed and transition forest of Southern Nigeria; it belongs to the family *Sterculiaceae* while the common names are monkey kola (English); *obi-edun* (Yoruba, Nigeria); *mba utong-ita* (Ibibio, Nigeria) and *ananse adodowa* (Ghana);

The plant is yet to be comprehensively studied, but the leaves, twigs, flowers, fruit follicles and the bark are used in folk medicine to prepare a tonic used as a remedy for dysentery, coughs, diarrhoea, vomiting and chest complaints. Odugbemi, (2006) [38] reported that the Yorubas of South Western Nigeria apply the leaves and fruits of *Cola millenii* in the treatment of ringworm, scabies, gonorrhoea, dysentery and opthalmia. Antimicrobial effect of ethanol extracts of *Cola millenii* against human isolated strains of *Staphylococcus aureus, Staphylococcus albus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* has also been reported (Sonibare *et al.,* 2009) [48].

Despite these extensive uses of *Cola millenii* K. Schum in folk medicine, not much is known about its chemical composition or pharmacology.

This study is therefore aimed at investigating the acute toxicity, identifying some phytochemicals present in this plant and the possible effects on haematological indices, lipid profile and biomarkers of cardiac and prostate dysfunction to establish the pharmacological importance as well as the safety/toxicity of this lesser known plant.

2. Materials and methods

Samples of ripe fruits of *Cola millenii* K. Schum were obtained from forest locations in Obong Itam village in Itu Local Government Area of Akwa Ibom State in Nigeria. The plant material was authenticated by a taxonomist Dr (Mrs.) M.E. Bassey of the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen with number 'Ubon, UUH 2687 'Itu' was deposited in the herbarium of the University of Uyo, Uyo, Nigeria.

The samples were washed with clean tap water to remove dirt on the fruits. After the fruits were kept for 2 hrs for the water to dry off, a sharp stainless steel knife was used to cut open the fruits, in order to remove the seed. The seeds were freed from the mesocarp and chopped into very small pieces using a stainless steel knife. They were then air-dried at room temperature (25 ^{0}C ± 2 ^{0}C) until a constant weight was obtained. After drying, the seeds were ground using a desk top blender and grinder (Model No: QBL-18L40, Turinar Corp, Shang-Hai, China) into fine particles and stored in a plastic container with screw cap.

The ground seeds (250 g) were soaked in 1 litre of 80% ethanol (Sigma-Aldrich, St. Louis, USA) for 24 hours. Thereafter, the clear extract was siphoned using a drip set into a stainless steel pan. Residues were re-extracted with additional 600 ml of 80% ethanol for a further 24 hours and filtered as described. The combined extract was evaporated at 45 ^{o}C in an open water bath until all the solvent was removed. The dried extract of the sample was refrigerated at 4 ^{0}C until required for use.

Chemical tests for the screening and identification of bioactive chemical constituents in *Cola millenii* K. Schum were carried out in the extracts using the standard procedures as described by Harborne (1973) [23], Trease and Evans (1989) [49] and Sofowara (1993) [47] and recorded (Table 1).

Wistar albino mice and rats obtained from the animal house, Biochemistry Department, University of Uyo, were used in this study. The animals were allowed to acclimatize for one week and maintained under standard laboratory conditions with rat chow (Vital Feeds, Jos, Plateau State, Nigeria) and water ad libitum. All animal experiments were carried out in line with the guidelines of Institutional Animal Ethical committee as approved by the graduate School, University of Uyo, Nigeria.

The Median lethal dose (LD_{50}) of *Cola millenii K. Schum* was determined using a total of 27 male albino mice weighing between 30 and 35 g in nine groups of three mice each by intraperitoneal (i.p) route adapting Lorke's method (Lorke, 1983) [31]. This involved intraperitoneal administration of a dose range (1000–6000 mg/kg body weight) of the extract intraperitoneally after being fasted overnight ('Staircase method'). The animals were observed for first 2 hours and then at 6th and 24th hour for physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. After 24 hours, the number of deceased mice was counted in each group; fraction and percentage of mortality were computed and recorded (Table 2). The LD_{50} was calculated as geometrical means of the maximum (most tolerable) dose producing 0% mortality (a) and the minimum (least tolerable) dose producing 100% mortality (b) using the formula; $LD_{50} = \sqrt{ab}$ (Lorke, 1983) [31].

Twenty eight (28) mature normal male *albino* rats of the Wistar strain weighing 151 – 195 g were used in this work. Animals in group I (control) received an equivalent volume (0.34 ml) of 20% Tween® 80 as placebo. The test groups II, III and IV received 150, 300 and 450 mg/kg body weight of extract respectively representing 1/10, 2/10 and 3/10 of the LD_{50} for a period of seven days (one week) by intraperitoneal route (i.p.). The extract was dissolved in 20% Tween® 80 vehicle and delivered in 0.16, 0.36 and 0.50 ml to group II, III and IV respectively.

3. Animal Sacrifice and Preparation of Sera for Analysis

All experimental animals were anaesthetized using chloroform fumes 24 hours after the last administration of the extract. Thereafter, the animals were sacrificed and blood samples for sera preparation collected by cardiac puncture into sterile plain tubes for which EDTA 0.77M (anticoagulant) had been added. However, no anticoagulant was added to blood samples for haematological analysis. Serum samples were extracted from the clotted blood into sterile plain tubes after centrifugation at 2000 rpm for 10 minutes using a bench top centrifuge (MSE Minor, England). The sera were stored in the refrigerator for analyses while the whole blood samples were used in determining

haematological indices. All biochemical analyses were carried out within 48 hours of sample collection.

4. Determination of biochemical parameters

All chemicals and reagents used for this research were of analytical grade. Absolute ethanol and Tween®80 were obtained from Sigma-Aldrich, St. Louis, USA. BC-23400 haematological analyzer (Shenzhen Mindray, Bio Medical Electronics Co., Ltd., China) was used in the determination of haematological indices. Lipid profile was determined by the enzymatic colorimetric methods using TECO diagnostic kits reagents (TECO Diagnostic, USA). The absorbance was measured using an Optima 3000 nano-UV/vis scanning spectrophotometer (Optima, USA). Assay kits for estimation of serum alanine amino transferase (ALT), alkaline phosphatase (ALP), aspartate amino transferase (AST), γ-glutamyl transferase (γ-GT), uric acid, Creatine Kinase (CK), CK-MB, Prostate specific antigen (PSA) and Testosterone ELISA kits were obtained from Teco Diagnostics, 1268 N. Lakeview Ave. Anahiem, CA 92807, USA. Quantification was carried out using a micro-plate reader (TECO Diagnostic, USA). Assay kits for the estimation of serum albumin and total protein were obtained from Randox Laboratories Ltd. 55 Diamond Road, Crumlin, Co. Antrim, UK. Steroid 5-alpha reductase 2 (SRD5a2) ELISA kits was be obtained from Uscn Life Science Inc. Wuhan 430056, P. R. China.

Statistical analysis was carried out using the SPSS 11.0 statistical software (SPSS Inc., Chicago, IL).

5. Results

The phytochemicals identified in ethanol extract of *Cola millenii* seed include: alkaloids, terpenes, cardiac glycosides and saponins (Table 1). The effects of ethanol extract of *Cola millenii* seed on acute toxicity of albino mice (Table 2); haematological indices (Table 3), lipid profile (Fig.1), cardiac enzymes (Fig.2), liver enzymes (Fig.3), PSA, testosterone and 5-α reductase (Fig.4), albumins, total proteins and uric acid (Fig.5), liver, kidney and heart weight (Fig.6) are presented.

6. Acute toxicity (LD_{50}) determination

The LD_{50} from acute toxicity study carried out using albino mice in this investigation was calculated to be 2645.75 mg/kg body weight. Table 2 shows the percentage mortality in the acute toxicity study.

7. Biochemical parameters

The effects of exposure of male Wistar albino rats to *Cola millenii* seed extract (concentrations: 150, 300 and 450 mg/kg body weight for 7 days), selected based on LD_{50} of 2645.75 mg/kg body weight caused significant ($p<0.05$) dose dependent decrease in TC, TG, LDL-C and an increase in HDL cholesterol compared with the normal control (Fig.1). The results also revealed a significant decrease in CK in all the test groups compared to the control, decrease in CK-MB activity which was significant only in group 3, decrease in LDH-L activity which was significant in groups 3 and 4. There was a non-significant decrease in AST activity, decrease in ALT activity which was significant only in groups 2 and 3, and a decrease in ALP activity which was significant only in groups 2 and 4. However, GGT activity increased significantly in all the test groups (Fig. 2 and Fig. 3). Results of prostate biomarkers showed a significant decrease in PSA levels in all the test groups and no significant changes in testosterone and 5-α reductase levels (Fig. 4). There was significant decrease in total protein and uric acid levels while albumin level remained unchanged (Fig.5). The kidney and liver weights increased significantly in groups 2 and 3 while the heart weight also decreased significantly in groups 2 and 3 (Fig. 6). Equally, haemoglobin, PCV, and MCV decreased significantly while platelets counts increased significantly. There were no significant changes in RBC, MCH, MCHC, lymphocytes as well as neutrophils counts (Table 3).

Table 1: Phytochemical screening of ethanol extract of *Cola millenii* seed

Constituents	Results
Alkaloid	++
Terpenes	+
Cardiac glycosides	+++
Saponins	+
Flavonoids	-
Tannins	-
Phlobatannins	-
Phenols	-
Anthraquinones	-

+++ = strongly present, ++ = present in high concentration, + = present, but not in high concentration, - = absent or below detectable concentration

Table 2: Acute toxicity test (LD_{50}) of ethanol extract of *Cola millenii* seed

S/N	Dosage (mg/kg body weight)	Fraction of death	Percentage (%) of death
1.	1000	0/3	0.00
2.	1500	0/3	0.00
3.	2000	0/3	0.00
4.	2500	2/3	66.67
5.	3000	2/3	66.67
6.	3500	3/3	100.00
7.	4000	3/3	100.00
8.	5000	3/3	100.00
9.	6000	3/3	100.00

Using Lorke's method;

$LD_{50} = \sqrt{axb}$

Where a = the maximum (most tolerable) dose producing 0 % mortality, and

b = the minimum (least tolerable) dose producing 100 % mortality

$LD_{50} = \sqrt{2000x3500}$

= 2645.75mg/kg

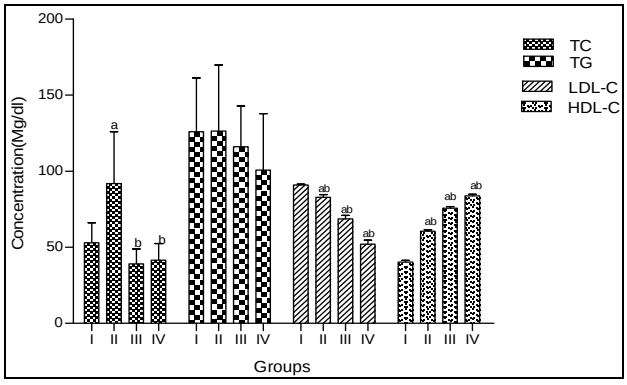


Fig 1: Effect of ethanol extract of *Cola millenii* seed on lipid profile of male albino Wistar rats

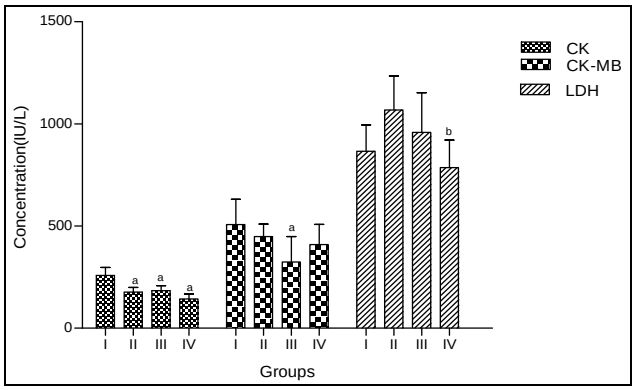


Fig 2: Effect of ethanol extract of *Cola millenii* seed on cardiac enzymes of male albino Wistar rats

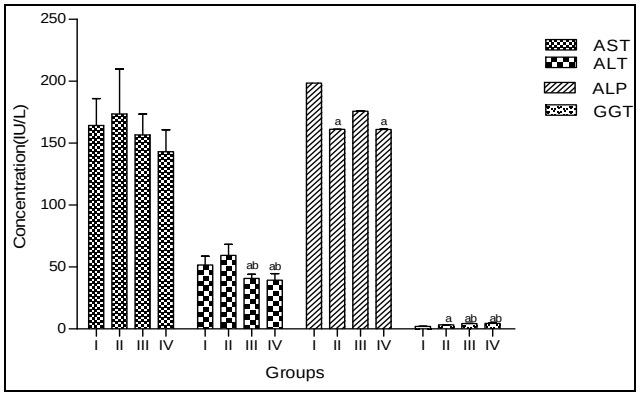


Fig 3: Effect of ethanol extract of *Cola millenii* seed on liver enzymes of male albino Wistar rats

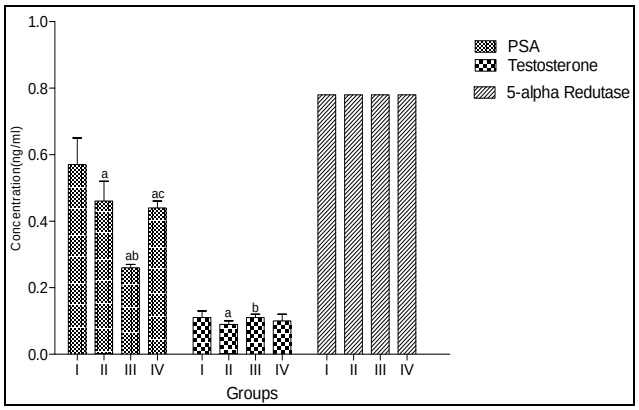
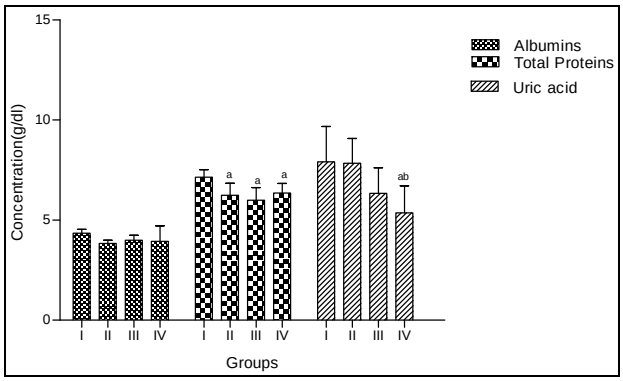


Fig 4: Effect of ethanol extract of *Cola millenii* seed on prostate functions of male albino Wistar rats



Note: Values of uric acid plotted was multiplied by 1000

Fig 5: Effect of ethanol extract of *Cola millenii* seed on serum albumins, total proteins and uric acid level of male albino Wistar rats

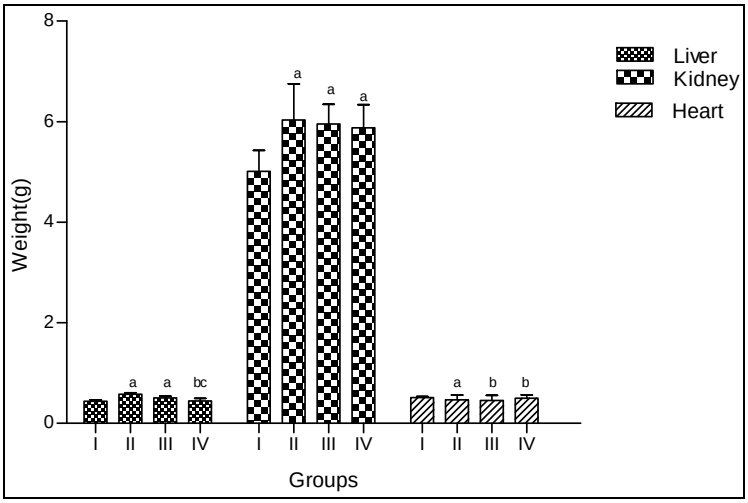


Fig 6: Effect of ethanol extract of *Cola millenii* seed on liver, kidney and heart weight of male albino Wistar rats

Table 3: Effect of ethanol extract of *Cola millenii* seed on hematological parameters of male albino Wistar rats.

Group	RBC x 10^6(µL)	HB (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBC x 10^3 (µL^{-1})	Lym (%)	Neut (%)	PLAT x 10^3 (µL)
I	7.47 ± 0.30	13.24 ± 0.44	40.87 ± 2.42	54.65 ± 1.94	17.71 ± 0.51	32.45 ± 1.13	12.06 ± 1.39	73.24 ± 7.39	23.74 ± 7.07	1158.14 ± 239.70
II	7.37 ± 0.52	13.45 ± 0.73	42.12 ± 2.50	57.15 ± 1.70[a]	18.29 ± 0.74	31.95 ± 1.05	10.57 ± 1.72	76.61 ± 10.35	20.05 ± 9.19	1124.57 ± 103.43
III	7.04 ± 0.18	12.37 ± 0.44[b]	37.92 ± 1.29[b]	53.84 ± 1.44[b]	17.55 ± 0.48	32.63 ± 0.60	11.41 ± 2.68	69.94 ± 4.47	25.92 ± 4.21	1429.42 ± 72.33[a,b]
IV	6.93 ± 0.79	12.25 ± 1.07[b]	37.07 ± 4.23[b]	53.51 ± 0.93[b]	17.77 ± 0.68	33.16 ± 1.32	14.04 ± 1.78[b]	70.82 ± 8.33	24.90 ± 8.24	1462.85 ± 50.69[a,b]

Values are expressed as Mean ± S.D, n = 6; a = p<0.05 (compared with control), b = p<0.05 (compared with group II

8. Discussion

In this study, we examined the effects of the ethanol extract of *Cola millenii* seed on some biochemical indices of Wistar albino rats. We also investigated the phytochemicals and the effects of ethanol extract of *Cola millenii* seed on acute toxicity using the albino mice model as an attempt to ascertain its medicinal significance.

The result of phytochemical screening (Table 1) shows that alkaloids, terpenes, saponins and cardiac glycosides were present in ethanol extract of *Cola millenii* seed. This finding agrees with the report of Giwa *et al.,* (2012) [21]. These phytochemicals according to Savage, (1993) [43] exhibit structure related biological, pharmacological and biochemical actions. Alkaloids, terpenes, cardiac glycoside, saponins possess various medicinal importance such as anti-HIV, anti-plasmodial, anti-diarrheal, anti-septic, anti-bacterial, anti-viral, anti-inflammatory, anti-microbial, hypoglycemic, antioxidant, analgesic and hepatoprotective properties (Sofowora, 1993; Evans, 2005; Ebana, Ellong and Owana, 2005; Cushnie and Lamb, 2005) [47, 18, 14, 11].

The result of the present study has shown that ethanol extract of *Cola millenii* seed has an intraperitoneal LD_{50}: (mice) of 2645.75 mg/kg body weight (Table 2). Jigam *et al.,* (2009) [26] reported an intraperitoneal LD_{50} (mice) of 3000 mg/kg body weight of the leaf extracts of *Lippia multiflora mold* as being at a high safety level. Therefore *Cola millenii* can be considered to be relatively safe and may be used for ethno-medical purposes.

The result of the effect of ethanol extract of *Cola millenii* seed on lipid profile (Fig.1) suggest that the extract may possess cardio-protective properties and may be useful in the management of coronary heart diseases (CHDs). The results of the effect of ethanol extract of *Cola millenii* seed on cardiac enzymes CK, CK-MB and LDH of male albino Wistar rats (Fig.2) is an indication that the extract may possess cardio-protective properties. This is in agreement with the report on the cardio-protective effects of ethanolic extract of *Litsea deccanensis* (ELD) against isoproterenol-induced myocardial infarction in rats (Kumar, Kannan and Quine (2011) [27, 28].

The result of the effect of ethanol extract of *Cola millenii* seed on liver enzymes of male albino Wistar rats (Fig. 3) suggests the hepato-protective potential of the extract which might be due to its antioxidants property as a result of the presence of bioactive constituents like alkaloid (Chukwudi, Simeon and Aguiyi, 2011) [10]. The suppression of liver enzymes to significant amounts could be due to the enhanced suppressive effect displayed by some components in ethanol extract of *Cola millenii* seed. Probably, a chemical component in the ethanol extract of *Cola millenii* seed is stabilizing the integrity of the cell membrane, keeping the membrane intact and the enzymes enclosed (Muthu and Krishnamoorthy, 2011, Chukwudi *et al.,* 2011) [34, 10]. However, elevated doses of the extract may have resulted in mild loss of membrane structure and integrity because of onset of lipid peroxidation leading to the elevated levels of GGT (Singh and Gupta 2011) [45].

PSA are widely used biological marker for prostate cancer, prostatitis, and benign prostatic hyperplasia (BPH). The result of the effect of ethanol extract of *Cola millenii* seed on indices of prostate dysfunction (Fig. 4) is an indication of its potential therapeutic value in inflammatory conditions such as BPH, PC, and protasis. The unchanged level of 5-alpha reductase in the experimental animals when compared with the control is a step further to confirm the PSA result in this investigation. The implication is that ethanol extract of *Cola millenii* seed may have blocked alpha adrenergic receptors for steroid 5-alpha reductase or had no effect at all on these cells, resulting in the non-synthesis of 5-alpha reductase, hence, the observed unchanged levels. 5-alpha reductase functions in the conversion of testosterone to a more active form called dihydrotestosterone (DHT) and could be inhibited by phenolic compounds (Kumar *et al.,* 2011) [27, 28]. Since 5- alpha reductase synthesis has been inhibited, the conversion of testosterone to DHT is also inhibited with a tendency towards the accumulation of testosterone which was observed by the significant ($p<0.05$) increase in testosterone levels in experimental animals of test group III. This conclusion is in agreement with an earlier report on acute toxicity, biochemical and haematological study of *Aframomum melegueta* (Akpanabiatu *et al.,* 2013) [6]. Extracts of other plants materials such as saw palmetto, pomegranate, flax seed, nettles, grape seed, pine bark and green tea extracts, either singly, in combination, or in supplementation with vitamin E, selenium, vitamin C and coenzyme Q have been reported to have reduced PSA levels significantly and are therefore used for the treatment of benign prostatic hyperplasia (BPH) with possible reduction of the risk of prostate cancer (Fagelman and Lowe, 2001; Amory *et al.,* 2007) [19, 7].

The significant decrease in the haematological parameters Hb, PCV, and MCV suggests that ethanol extract of *Cola millenii* seed at the dosage used may have suppressed haematopoiesis due to the presence of saponin, which has been reported to reduce haematological parameters probably due to lysis of blood cells or suppression of blood cell synthesis (Schneider, Sheidt and Brietmaier, 2003) [44].

The decreased levels of uric acid (Fig. 5) suggest its possible free radical scavenging activity (Lawson-Evi *et al.,* 2011) [30].

The reduction of serum levels of total protein is an indication that some bioactive components of the extract may have caused stress-mediated mobilization of protein to cope with the detrimental condition so imposed (Adedapo *et al.,* 2009) [1]. Protein mobilization is one of the strategies employed to meet the energy required for biotransformation and excretion of toxicants (Adedapo, Mogbojuri and Emikpe, 2009) [1]. Significant decrease in total protein is mainly due to increase in messenger RNA degradation which is a possible cause for hypoalbuminemia (Metwally *et al.,* 1990) [33].

Changes in body or organ weight are used as a valuable indicator of toxicity of a compound or extract preparation (Grance *et al.,* 2008) [22]. In the present study, result of the effect of ethanol extract of *Cola millenii* seed on kidney and liver weights (Fig. 6) are indications of selective toxicity (Okwu, 2005) [40]. The decrease in the heart weight is an indication of the absence of inotropic agents in the ethanol extracts of *Cola millenii* seed, hence a potential for cardioprotection.

9. Conclusion

We conclude that ethanol extract of *Cola millenii* seed may possess non-cytotoxic, cardio-protective and hepato-protective properties and may provide bioactive agent(s) for biopharmaceutical applications. It may also be useful in the management of benign prostate hyperplasia because of inhibition of 5-alpha reductase. However, the extract should be used with moderation as it is capable of causing reduction in Hb and PCV. More investigations on this plant seed extract are ongoing.

10. References

1. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety

Evaluations of the Aqueous Extract of the Leaves of *Moringa oleifera* in Rats. Journal of Medicinal Plants Research. 2009; 3(8):586-591.
2. Akpanabiatu M, Umoh I, Enyong E, Edet E, Uboh F. Influence of *Rauwolfia vomitoria* Root Bark on Cardiac Enzymes of Normal Wistar Albino Rats. Recent Programme on Medicinal Plant Research. 2006; 14:273-278.
3. Akpanabiatu MI, Umoh IB, Edet EE, Ekanem T, Ukaffia S, Ndem JI. Interaction of *Rauwolfia vomitoria* root bark extract with vitamin A on rats myocardium: serum liver marker enzymes. Indian Journal of Clinical Biochemistry. 2009a; 24:241-244.
4. Akpanabiatu MI, Uboh FE, Ekanem TB, Umoh IB, Eyong EU, Ukafia S. Effect of interaction of *Rauwolfia vomitoria* root bark extract with vitamin E on rats' liver enzymes. Turkish Journal of Biology. 2009b; 33:189-194.
5. Akpanabiatu M, Otitoju O, Edet E, Ndem J, Uwah A, Ufot F. Vitamin E Supplementation with *Rauwolfia Vomitoria* Root Bark Extract Improves Hematological Indices. North American Journal of Medical Sciences. 2012; 4(2):86-89.
6. Akpanabiatu MI, Ekpo ND, Ufot UF, Udoh NM, Akpan EJ, Etuk EU. Acute toxicity, biochemical and haematological study of Aframomum melegueta seed oil in male Wistar albino rats. Journal of Ethnopharm. 2013; 150:590-594.
7. Amory JK, Wang C, Swerdloff SR, Anawalt BD, Matsumoto AM, Bremner WJ. The Effect of 5α-Reductase Inhibition with Dutasteride and Finasteride on Semen Parameters and Serum Hormones in Healthy Men. Journal of Clinical Endocrionology and Metabolism. 2007; 92:1659-1665.
8. Bisong SA, Brown R, Osim EE. Comparative effects of Rauwolfia vomitoria and chlorpromazine on locomotor behavior and anxiety in mice. Journal of Ethnopharmacology. 2012; 132:334-339.
9. Bolk J. Natural Compounds in Cancer Therapy. Oregon Medical Press. 2001.
10. Chukwudi NH, Simeon O, Aguiyi CJ. Analysis of Some Biochemical and Haematological Parameters for *Mucuna Pruriens* (DC) Seed Powder in Male Rats. Pakistan Journal of Pharmaceutical Sciences. 2011; 24(4):523-526.
11. Cushnie TPT, Lamb AJ. Antimicrobial Activity of Flavonoids. International Journal of Antimicrobial Agents. 2005; 26:343-356.
12. Demo M, Oliva M, de las M, Lopez ML, Zunino MP, Zygadlo JA. Antimicrobial activity of essential oils obtained from aromatic plants of Argentina. Pharmaceutical Biology. 2005; 42:129-134.
13. Denmeade SR, Isaacs JT. Development of prostate cancer treatment: the good news. Prostate. 2004; 58:211-224.
14. Ebana MC, Ellong A, Owona D. L'amblyopie chez le Strabique en Milieu Camerounais. Bulletin of the Belgian Society of Ophthalmology. 2005; 297:39-44.
15. Edem D. Haematological and Histological Alterations Induced in Rats by Palm Oil – Containing Diets. European Journal of Scientific Research. 2009; 32(3):405-418.
16. Efferth T. Molecular pharmacology and pharmacogenomics of artemisinin and its derivatives in cancer cells. Current Drug Targets. 2006; 7:407-421.
17. Essien EU, Esenowo GJ, Akpanabiatu MI. Lipid composition of lesser known tropical seeds. Plant Food and Human Nutrition. 1995; 48:135-140.
18. Evans WC. Trease and Evans Pharmacognosy. 15th edition, Elsevier, A Division of Reed Elsevier India Private Limited, New Delhi, India. 2005, 20-22.
19. Fagelman E, Lowe FC. Saw Palmetto Berry as a Treatment for BPH. Reviews in Urology. 2001; 3(3):134-138.
20. Farnsworth N, Soejarto D. Potential Consequences of Plant Extinction in the United States on the Current and Future Availability of Prescription Drugs. Society for Economic Botany. 1985; 39:231-240.
21. Giwa OE, Onileke FO, Adesina IA, Adebote VT. Phytochemicals and Antimicrobial Properties of Seed and Pulp of Monkey Cola (*Cola millenii*) on some Selected Clinical and Food Borne Isolate. International Journal of Applied Biology and Pharmaceutical Technology. 2012; 3(3):390-400.
22. Grance SRM, Teixeira MA, Leite RS, Guimar˜aes EB, Siqueira JM, Oliveira-Filiu WF. *Baccharis trimera*: Effect of Haematological and Biochemical Parameters and Hepatorenal Evaluation in Pregnant Rats. Journal of Ethnopharmacology. 2008; 117:28-33.
23. Harborne J. Phytochemical Methods. In: Harborne, J. B. (Ed.), A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall. 1973, 279.
24. Ifere GO, Abebe F, Ananaba GA. Prostate Cancer Gene Expression Marker1 (PCGEM1): A Patented Prostate-Specific Non-Coding Gene and Regulator of Prostate Cancer Progression. Recent Patents on DNA and Gene Sequences. 2009; 3(3):151-163.
25. Itharat A, Ooraikul B. Research on Thai Medicinal Plants for Cancer Treatment. Advances in Medicinal Plants Research. 2007; 81:1213-1215.
26. Jigam AA, Akanya HO, Ogbadoy EO, Dauda BE, Evans EC. *In vivo* Antiplasmodial, Analgesic and Anti-Inflammatory Activities of the Leaf Extract of *Lippia multiflora* Mold. Journal of Medicinal Plants Research. 2009; 3(3):148-154.
27. Kumar T, Chaiyasut C, Rungseevijitprapa W, Suttajit M. Screening of Steroid 5α-Reductase Inhibitory Activity and Total Phenolic Content of Thai Plants. Journal of Medicinal Plants Research. 2011; 5(7):1265-1271.
28. Kumar PB, Kannan MM, Quine DS. *Litsea deccanensis* Ameliorates Myocardial Infarction in Wistar Rats: Evidence from Biochemical and Histological Studies. Journal of Pharmacology. 2011; 3(4):287-296.
29. Latha S, Kannabiran K. Antimicrobial Activity and Phytochemicals of *Solanum trilobatum*. African Journal of Biotechnology. 2006; 5(23):2402-2404.
30. Lawson-Evi P, Eklu-Gadegbeku K, Agbonon A, Aklikokou K, Creppy E, Gbeassor M. Antidiabetic Activity of *Phyllanthus amarus* Schum and Thonn (Euphorbiaceae) on Alloxan Induced Diabetes in Male Wistar Rats. Journal of Applied Science. 2011; 11:2968-2973.
31. Lorke D. A New Approach to Practical Acute Toxicity Testing. Archives of Toxicology. 1983; 54:275-287.
32. Lucy H, Edgar J. Medicinal Plants: A Re-Emerging Health Aid. Electronic Journal of Biotechnology. 1999; 2(2):1-15.
33. Metwally AA, Janku I, Kemper F, Khayyal MT, Ebeid FA, Botros SS. Effect of Schistosomiasis Infection on the Cleavance of Phenazone in Mice. Arzneimittel Forschung – Drug Research Journal. 1990; 40:206-209.

34. Muthu K, Krishnamoorthy P. Evaluation of Androgenic Activity of *Mucuna pruriens* in Male Rats. *African* Journal of Biotechnology. 2011; 10(66):15017-15019.
35. Nelson R. The Comparative Clinical Pharmacology and Pharmacokinetics of Vindesine, Vincristine and Vinblastine in Human Patients with Cancer. Medical and Pediatric Oncology Journal. 1982; 10:115-127.
36. Nwankpa P, Eteng MU, Akpanabiatu MI, Oze G, Nwanjo HU. Effects of *Phyllanthus amarus* on serum lipid profile and oxidative stress status in *Salmonellae typhi* infested Wistar rats. Journal of Natural Product Plant Resources. 2012; 2:574-578.
37. Obasi N, Egbuonu A, Ukoha P, Ejikeme P. Comparative Phytochemical and Antimicrobial Screening of some Solvent Extracts of *Samanea saman* (Fabaceae or Mimosaceae) Pods. African Journal of Pure and Applied Chemistry. 2010; 4(9):206-212.
38. Odugbemi T. Medicinal Plants by Species Names: Outlines and Pictures of Medicinal Plants from Nigeria.10. University of Lagos Press. 2006, 158.
39. Okigbo R, Eme U, Ogbogu S. Biodiversity and Conservation of Medicinal and Aromatic Plants in Africa. Biotechnology and Molecular Biology Reviews. 2008; 3(6):27-34.
40. Okwu D. Phytochemicals, Vitamins and Mineral Contents of Two Nigeria Medicinal Plants. International Journal of Molecular Medicine and Advance Sciences. 2005; 1(4):375-381.
41. Pucar D, Sella T, Schder H. The role of imaging in the detection of prostate cancer cancer local recurrence after radiation therapy and surgery. Current Opinion Urology. 2008; 18:87-97.
42. Richter E, Srivastava S, Dobi A. Androgen receptor and prostate cancer. Prostate Cancer and Prostatic Diseases. 2007; 10:114-118.
43. Savage GP. Saponins. In: Macre, R., Robinson, R. K. and Sadler, M. J (Eds.), Encyclopedia of Food Science, Food Technology and Nutrition. London, Academic Press. 1993, 3998-4001.
44. Schneider CR, Sheidt K, Brietmaier E.: Four New Pregnant Glycosides from *Gongronema latifolium* (Asclepiadaceae). Journal Parkische Chemistry Chenisker-Zutung. 2003; 353:532-536.
45. Singh D, Gupta RS. Hepatoprotective Activity of Methanol Extract of *Tecomella undulata* against Alcohol and Paracetamol Induced Hepatotoxicity in Rats. Life Sciences and Medicine Research, (LSMR). 2011; 26:15-19.
46. Soetan KO, Aiyelaagbe OO. The Need for Bioactivity-Safety Evaluation and Conservation of Medicinal Plants - A Review. Journal of Medicinal Plants Research. 2009; 3(5):324-328.
47. Sofowora A. Medicinal Plants and Traditional Medicine in Africa, 2nd ed., Ibadan, Nigeria: Spectrum Books Limited (Publisher). 1993, 134 - 156.
48. Sonibare M, Micheal O, Oyedokun O, Oluwadayo O. Phytochemical and Antimicrobial Studies of Four Species of *Cola* Schott & Endl. (Sterculiaceae). African Journal of Traditional, Complementary and Alternative medicines. 2009; 6(4):518-525.
49. Trease G, Evans W. A Textbook of Pharmacognosy, 13th ed., London: Bailliere Tindall Ltd. 1989, 19-21.
50. United Nations Educational, Scientific and Cultural Organization (UNESCO). Promotion of Ethnobotany and the Sustainable use of Plant Resources in Africa. FIT/504-RAF-48 Terminal Report: Paris, France. 1998; 60-62.
51. Wang X, Yuan S, Wang J, Lin P, Liu Y, Zhang J. *et al.* Anticancer activity of litchi fruit pericarp extract against human breast cancer *in vitro* and *in-vivo*. Toxicology and Applied Pharmacology. 2006; 215:168-178.
52. World Health Organization (WHO). Resolution – Promotion and Development of Training and Research in Traditional Medicine. WHO Document No. 1977, 30-49.
53. Yakubu MT, Afolayan AJ. Effect of Aqueous Extract of *Bulbine natalensis* Bark and Stem on Haematological and Serum Lipid Profile of Male Wistar Rats. Indian Journal of Experimental Biology. 2009; 47:283-288.
54. Farnsworth NR. The role of medicinal plants in drug development. In: Natural products and drugs developments. Eds. Ballier, Tindall and Cox, London, 1984, 9-98.