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

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**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, phytochemical 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 LD50 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. 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 hepatoprotective 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 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-edin (Yoruba, Nigeria); mba utong-ita (Ibibio, Nigeria) and ananse adadova (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, temperature (25°C ± 2°C) until a constant weight was obtained. The samples were washed with clean tap water to remove dirt from the mesocarp and chopped into very small pieces using a stainless steel knife. They were then air-dried at room temperature (25°C ± 4°C) in an open water bath until all the solvent was removed. The dry extract of the sample was refrigerated at 4°C until required for use.

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 °C ± 2 °C) until a constant weight was obtained. After drying, the seeds were ground using a desk top blender and grinder (Model No: QBL-18L40, Turinlar 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 °C in an open water bath until all the solvent was removed. The dried extract of the sample was refrigerated at 4°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) [33], Trease and Evans (1989) [40] and Sofowara (1993) [42] and recorded (Table 1). Wistar albino mice and rats obtained from the animal house, Biochemistry Department, University of Uyo, Nigeria, 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 (LD50) 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 LD50 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 LD50 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 (TECO Diagnostic, USA). The absorbance was measured using an Optima 3000 nano-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 Tecno Diagnostics, 1268 N. Lakeview Ave. Anaheim, 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 Usn 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 5α reductase (Fig.4), albumins, total proteins and uric acid (Fig.5), liver, kidney and heart weight (Fig.6) are presented.

6. Acute toxicity (LD50) determination
The LD50 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 LD50 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 5α 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

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>++</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
</tbody>
</table>

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

### Table 2: Acute toxicity (LD50) of ethanol extract of *Cola millenii* seed

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

Using Lorke’s method;

$$LD_{50} = \sqrt{a \times b}$$

Where $a$ = the maximum (most tolerable) dose producing 0 % mortality, and $b$ = the minimum (least tolerable) dose producing 100 % mortality

$$LD_{50} = \sqrt{2000 \times 3500} = 2645.75$$

![Fig 1: Effect of ethanol extract of *Cola millenii* seed on lipid profile of male albino Wistar rats](image)
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

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC x 10⁶(µL)</th>
<th>HB (g/dl)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>WBC x 10⁸ µL⁻¹</th>
<th>Lym (%)</th>
<th>Neut (%)</th>
<th>PLAT x 10³ (µL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.47 ± 0.30</td>
<td>13.24 ± 0.44</td>
<td>40.87 ± 2.42</td>
<td>54.65 ± 1.94</td>
<td>17.71 ± 0.51</td>
<td>32.45 ± 0.13</td>
<td>12.06 ± 1.39</td>
<td>73.24 ± 7.39</td>
<td>23.74 ± 7.07</td>
<td>1158.14 ± 239.70</td>
</tr>
<tr>
<td>II</td>
<td>7.37 ± 0.52</td>
<td>13.45 ± 0.73</td>
<td>42.12 ± 2.50</td>
<td>57.15 ± 1.70</td>
<td>18.29 ± 0.74</td>
<td>31.95 ± 1.05</td>
<td>10.57 ± 1.72</td>
<td>76.61 ± 10.35</td>
<td>20.05 ± 9.19</td>
<td>1124.57 ± 103.43</td>
</tr>
<tr>
<td>III</td>
<td>7.04 ± 0.18</td>
<td>12.37 ± 0.44</td>
<td>37.92 ± 1.29</td>
<td>53.84 ± 1.44</td>
<td>17.55 ± 0.48</td>
<td>32.63 ± 0.60</td>
<td>11.41 ± 2.68</td>
<td>69.94 ± 4.47</td>
<td>25.92 ± 4.21</td>
<td>1429.42 ± 72.33</td>
</tr>
<tr>
<td>IV</td>
<td>6.93 ± 0.79</td>
<td>12.25 ± 1.07</td>
<td>37.07 ± 4.23</td>
<td>53.51 ± 0.93</td>
<td>17.77 ± 0.68</td>
<td>33.16 ± 1.32</td>
<td>14.04 ± 1.78</td>
<td>70.82 ± 8.33</td>
<td>24.90 ± 8.24</td>
<td>1462.85 ± 50.69</td>
</tr>
</tbody>
</table>

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 glycosides, saponins possess various medicinal importance such as anti-HIV, anti-plasmodial, anti-diarrheal, anti-septic, anti-bacterial, antiviral, anti-inflammatory, anti-microbial, hypoglycemic, antioxidant, analgesic and hepatoprotective properties (Sofowora, 1993; Evans, 2005; Ebana, Ellong and Owana, 2005; Cushnie and Lamb, 2005) [14, 11].

The result of the present study has shown that ethanol extract of *Cola millenii* seed has an intraperitoneal LD₅₀ (mice) of 2645.75 mg/kg body weight (Table 2). Jigam et al., (2009) [26] reported an intraperitoneal LD₉₀ (mice) of 3000 mg/kg body weight of the leaf extracts of *Lippia multiflora* as being at a high safety level. Therefore *Cola millenii* can be considered to be relatively safe and may be used for ethnomedical 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 cause 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 hepatoprotective 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) [14, 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) [49].

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 prostatitis. 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* (Akpanabiata 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 haematopoesis 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, Shieldt 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 hepatoprotective 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


