Evaluation of Acute and Subchronic Toxicities of Class Bitters®: A Polyherbal Formula in Male Wistar Albino Rats

Kingsley C. Patrick-Iwuanyanwu, Denning E. Chinaka and Blessing B. Gboelo
Department of Biochemistry, Toxicology Unit, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria

Abstract: Objective: This study was carried out to evaluate the acute and subchronic toxicities of Class Bitters® (CB)-a polyherbal formula prepared with Monia whitei, Khaya senegalensis, Capparis erythrocarpus, Thornngia sanguinea and Xylophy aethiopica in male Wistar albino rats. Materials and Methods: Administration of 2000 mg kg⁻¹ b.wt., of CB in an acute toxicity study recorded no mortality in rats within the observable period of 14 days. However, treatment of rats with CB at doses of 500 and 1000 mg kg⁻¹ b.wt., respectively for 9 weeks in a sub-chronic study showed an increase in the daily consumption of feed and fluid intake in experimental rats. Results: A progressive increase in body weight was observed in rats administered with 500 and 1000 mg kg⁻¹ b.wt., respectively during the exposure period. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP) activities and cholesterol level increased significantly (p<0.05) in rats treated with 500 and 1000 mg kg⁻¹ b.wt., respectively whereas a significant (p<0.05) increase in the level of Triglyceride was only observed in rats treated with 1000 mg kg⁻¹ b.wt. when compared with control. Total bilirubin and urea were significantly (p<0.05) decreased while Creatinine showed no significant change. Histological examination of the liver and testes showed normal architecture in control group whereas hepatocytes of rats treated with 500 and 1000 mg kg⁻¹ b.wt., respectively were characterized by chronic hepatitis. However, there were no observable pathological changes in the testes of rats in the experimental groups. Conclusion: These observations suggest that long term administration of CB may be toxic and its toxicity may be organ specific.

Key words: Acute toxicity, subchronic toxicity, hepatotoxicity, class bitters, histological examination

INTRODUCTION

Herbal medicine, also known as phytotherapy or phytomedicine has played a significant role in the prevention and treatment of various human ailments since time immemorial (Schulz et al., 2001; Usman et al., 2012). It encompasses all kinds of folk medicine, unconventional medicine and indeed, any kind of therapeutic method that has been continually passed from generation to generation (Brown, 2003). Since the use of herbal remedies in the treatment of diseases has recorded a huge success, it has therefore been employed in developing countries as an alternative to orthodox pharmacotherapy (Sofowora, 1989; Zhu et al., 2002). According to WHO (2008), about 80% of the world population rely on traditional medicine for primary health care and more than 30% of plant species have been used medicinally. In contrast to the use of synthetic drugs in modern medicine, the potential toxicity of the use of herbal remedies has not been fully investigated scientifically (Pierre et al., 2006).

The damaging effects of herbal remedies to the human body is generally considered to be lower than synthetic drugs and as such may be Generally Regarded As Safe (GRAS) (Alam et al., 2011). Because of the increasing use of herbal formulation in nutraceuticals, there is a compelling need for evaluation and standardization of medicinal plants (Ben-Arye et al., 2011; Haque and Haque, 2011). Herbal preparations could be contaminated with microbiological and foreign materials such as heavy metals, pesticide residues or even aflatoxins. Contaminants in herbal preparations may cause prominent health defects underscoring the claimed safety (Ogbomia et al., 2011). Therefore, an increase in the morbidity and mortality associated with the use of herbal or the so called traditional medicines have raised universal attention in the last few years (Wickramasinghe, 2006). Polyherbal medicines are administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage.
(Ogbonnia et al., 2011). The danger associated with the potential toxicity of such therapy and other poly-herbal therapies used over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from ingestion of medicinal herbs (Tedong et al., 2007). Class Bitters®(CB) is a poly-herbal formula produced by Classic Herbal Centre in Accra, Ghana. Ethnomedically, CB is taken three times daily for the treatment of diabetes mellitus, muscle pains, joint pains, backache, general body pains and sexual weakness. The present investigations were therefore carried out to determine the acute and subchronic toxicity profile of CB in male Wistar albino rats.

MATERIALS AND METHODS

Herbal sample: Five bottles of CB with the same batch number Bx/04/10 produced by Classic Herbal Centre, Accra, Ghana were used for this study. They were purchased from a local herbal drug retailer in Rumuola, Port Harcourt, Rivers State, Nigeria in August, 2011.

Acute toxicity test: Healthy male Wistar albino rats weighing between 140-160 g maintained under standard laboratory conditions were used for acute toxicity test according to The Organisation for Economic Cooperation and Development (OECD) guidelines 425 (OECD 2000 guideline). A total of ten animals were used which received a single oral-dose of 2000 mg kg⁻¹ b.wt. of CB. Animals were kept overnight fasting prior to drug administration by oral gavage. After administration of drug sample, food was withheld for further 3-4 h. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Daily observations on the changes in skin and fur, eyes and mucous membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes were noted (OECD, 2000).

Animals: A total of 30 male Wistar albino rats weighing between 140 to 160 g used this study were obtained from The Animal House of the Department of Biochemistry, University of Port Harcourt, Choba, Rivers State, Nigeria. The animals were kept singly in a cross-ventilated house and were fed with standard rat pellet and water ad libitum. The rats were acclimatized for 7 days.

Subchronic oral toxicity study: Thirty male Wistar albino rats were divided into three groups of 10 rats per group. Group 1 served as the control and received standard feed and distilled water only. Groups 2 and 3 received standard feed, distilled water and CB at doses of 500 and 1000 mg kg⁻¹ b.wt., respectively (Chandra et al., 2010). Administration of the extract was done orally, by means of a polythene cannula. Animals received their doses once a day for 9 weeks. They were observed daily for clinical signs of toxicity or pharmacological signs, throughout the period of study.

Food and water consumption: The amount of feed and water consumed were measured daily from the quantity of feed and water supply and the amount remaining after 24 h.

Measurement of body weight: The body weights of the animals were evaluated weekly and recorded using a sensitive balance (OECD, 1995).

Sample collection: At the end of the treatment period, the animals were weighed and sacrificed using cervical dislocation method. Blood samples were obtained by cardiac puncture using a 2 mL hypodermal syringe. The blood samples were introduced into clean dry anticoagulant free bottles. The anti-coagulant free bottles containing the samples were centrifuged at 3000 rpm for 10 min to separate serum from the packed cells. The serum obtained was collected into a clean dry sample bottle and used for the analysis of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Triglyceride, cholesterol, urea and creatinine. The levels of biochemical parameters were estimated using the Humazym MUV-test kits.

Histopathological study: A portion of the liver of all the rat groups was fixed in 10% buffered neutral formalin for 48 h followed by bovine solution for 6 h and then processed for paraffin embedding. By using a microtome, sections of 5 μm thickness were taken, processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin (Galigher and Kayloff, 1971) and subjected to histopathological examination.

Statistical analysis: Values are expressed as means±Standard deviation (SD). The results were analyzed statistically by Analysis of Variance (ANOVA) followed by Turkey Multiple Comparison Test. Significance was accepted at a p-value of 0.05.
RESULTS

The results of the acute toxicity study showed no mortality or physical changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000 mg kg\(^{-1}\) b.wt. of CB. Since none of the mentioned toxic signs and symptoms or mortality was observed in the animals at the above mentioned dose, 500 and 1000 mg kg\(^{-1}\) b.wt. of test drug were selected for the study. The results of daily feed and fluid intakes of the control and treated rats are shown in Table 1. Results from the study showed that after 9 weeks of administration of test drug, the rats treated with 1000 mg kg\(^{-1}\) b.wt. consumed more feed and also recorded the highest water intake as compared with control. The result of the study showed that sub-chronic administration of CB in treated groups had no effect on the normal growth of rats as compared to control. A progressive increase in body weight of rats was observed during the exposure period in groups administered 500 and 1000 mg kg\(^{-1}\) b.wt. when compared with control (Fig. 1). The effects of the result of CB on liver enzymes are shown in Table 2. Result from the study showed significant (p<0.05) increases in AST, ALP and ALT activities in the groups treated with CB with rats in the group administered 1000 mg kg\(^{-1}\) b.wt. showing the highest activity for AST, ALP and ALT when compared to control (Table 2). Administration of CB at doses of 500 and 1000 mg kg\(^{-1}\) b.wt. to experimental rats significantly (p<0.05) increased serum total protein and conjugated bilirubin, respectively whereas total bilirubin decreased significantly when compared with control (Table 3). The result of the effect of CB on Cholesterol, Triglyceride, Urea and Creatinine are shown in Table 4. Result of the study showed a significant (p<0.05) increase in the level of cholesterol in groups administered 500 and 1000 mg kg\(^{-1}\) b.wt. of CB when compared with control. However, rats in the group administered 500 and 1000 mg kg\(^{-1}\) b.wt. showed a significant (p<0.05) decrease in the level of Urea (Table 4). There was however no significant change in the level of creatinine among the groups (Table 4). Results of the histological examination of the liver and testes are shown in Fig. 2a-c. The result of the study on the liver in the control group showed normal architecture of hepatocytes with conspicuous nucleus and obvious sinusoids (Fig.2) whereas, hepatocytes of rats in the groups administered 500 and 1000 mg kg\(^{-1}\) b.wt. were characterized by chronic hepatitis (Fig. 2 and 3). The results of histological examination of rats testes showed normal testicular structure in all the experimental groups (Fig. 3a-c).

Table 1: Daily feed and water intake of CB exposed rats

<table>
<thead>
<tr>
<th>CB Conc. (mg kg(^{-1}))</th>
<th>Daily feed intake (g day(^{-1}))</th>
<th>Daily water intake (ml day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.94±3.28</td>
<td>44.09±4.80</td>
</tr>
<tr>
<td>500</td>
<td>36.77±2.82</td>
<td>47.59±4.73</td>
</tr>
<tr>
<td>1000</td>
<td>39.85±2.79</td>
<td>49.07±4.81</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD, n=10

Table 2: Effect of sub-chronic oral administration of CB on serum biochemical parameters of rats

<table>
<thead>
<tr>
<th>CB Conc. (mg kg(^{-1}))</th>
<th>AST (IU L(^{-1}))</th>
<th>ALP (IU L(^{-1}))</th>
<th>ALT (IU L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>122.8±34.76</td>
<td>259.8±44.93</td>
<td>82.5±12.55</td>
</tr>
<tr>
<td>500</td>
<td>146.7±29.20</td>
<td>358.9±51.51</td>
<td>88.0±13.90</td>
</tr>
<tr>
<td>1000</td>
<td>215.0±26.21</td>
<td>524.9±41.80</td>
<td>88.9±13.17</td>
</tr>
</tbody>
</table>

n=10, Values (Mean±SD) with different superscripts are considered to be significantly different at p<0.05

Table 3: Effect of subchronic oral administration of CB on total protein, conjugated bilirubin and total bilirubin in male Wistar albino rats

<table>
<thead>
<tr>
<th>CB Conc. (mg kg(^{-1}))</th>
<th>Total protein (mg dl(^{-1}))</th>
<th>Conjugated bilirubin (mg dl(^{-1}))</th>
<th>Total bilirubin (mg dl(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.6±9.30</td>
<td>5.6±4.05</td>
<td>11.8±0.75</td>
</tr>
<tr>
<td>500</td>
<td>81.2±13.54</td>
<td>6.5±6.09</td>
<td>7.6±3.08</td>
</tr>
<tr>
<td>1000</td>
<td>81.9±12.90</td>
<td>8.0±4.90</td>
<td>7.1±2.68</td>
</tr>
</tbody>
</table>

n=10, Values (Mean±SD) with different superscripts are considered to be significantly different at p<0.05

Table 4: Effect of subchronic oral administration of CB on cholesterol, triglycerides, urea and creatinine in male Wistar albino rats

<table>
<thead>
<tr>
<th>CB Conc. (mg kg(^{-1}))</th>
<th>Cholesterol (mmol L(^{-1}))</th>
<th>Triglyceride (mmol L(^{-1}))</th>
<th>Urea (mmol L(^{-1}))</th>
<th>Creatinine (mmol L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.46±0.20</td>
<td>1.69±0.37</td>
<td>9.2±1.15</td>
<td>95.7±8.25</td>
</tr>
<tr>
<td>500</td>
<td>3.28±0.38</td>
<td>1.75±0.40</td>
<td>8.38±0.88</td>
<td>104.9±7.26</td>
</tr>
<tr>
<td>1000</td>
<td>3.38±0.58</td>
<td>2.03±0.32</td>
<td>7.55±1.67</td>
<td>107.1±1.98</td>
</tr>
</tbody>
</table>

n=10, Values (Mean±SD) with superscripts are considered to be significantly different at p<0.05

![Fig. 1: Effect of CB on body weight changes in control and treated rats](image-url)
Fig. 2(a-c): Rat liver (a) control with normal architecture and chronic hepatitis in rats administered, (b) 500 mg kg\(^{-1}\) and (b) 1000 mg kg\(^{-1}\) b.wt., CB

Fig. 3(a-c): Rat testes of control group (a) and rats administered 500 and 1000 mg kg\(^{-1}\) b.wt., respectively and (b and c) showing normal testicular structure

**DISCUSSION**

The uses of herbs require good knowledge of the toxicity dosage, purity, suitable extraction solvent and adverse effects (Murray, 1998). The result of the present study on the acute toxicity study of CB showed no mortality or physical changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000 mg kg\(^{-1}\) b.wt. This observation may be an indication that the drug could be considered relatively safe especially when administered orally where absorption may not be complete due to inherent factors limiting absorption in the gastrointestinal tract (Shatoor, 2011). Subchronic administration of CB had no negative effect on the normal growth of rats suggesting that it did not possibly cause any alterations in carbohydrate, protein or fat metabolism in these experimental animals. It also shows that CB did not adversely interfere with the nutritional benefits (e.g., weight gain, stability of appetite) expected of animals that are continually supplied with food and water ad libitum (Anigbu et al., 2005). Changes in body weight have been used as an indicator of adverse effect of drugs and chemicals (Mukinda and Syce, 2007).

The increased level of AST, ALP and ALT is conventional indicator of liver injury (Shah et al., 2011). Ramasah (2011) reported that analysis of some basic liver enzymes in serum or plasma can be used to indirectly assess the integrity of tissues after being exposed to certain pharmacological agents. However, the administration of 500 and 1000 mg kg\(^{-1}\) b.wt. of CB elevated the activities of these serum markers. This elevation may be attributed to severe hepatocellular injury. Liver injury has been attributed to xenobiotics (Omiecinski et al., 2011). Results from the study showed that AST concentrations were consistently higher than ALT levels. This observation is expected since body cells
contain more AST than ALT (Mayne, 1996). High AST levels indicate liver damage (Crook, 2006). However, ALP is excreted normally via bile by the liver. Liver injury due to toxins can result in defective excretion of bile by the hepatocytes which are reflected as their increased levels in serum (Rajesh and Latha, 2004). The significant increase in ALP activity by CB shows possible bile obstruction or cholestasis occurred at the dose levels tested, since a rise in serum ALP is usually a characteristic finding in cholestatic liver disease (Mythilypriya et al., 2007; Wasan et al., 2001). However, ALT concentration showed no significant difference. The high activities of serum markers observed in this study corroborates the findings of Sule et al. (2008) who, reported that the stem bark of Khaya senegalensis (one of the active ingredients of CB) at a high concentration elevated the activities of AST, ALT and ALP. The increase in the level of serum enzymes may be attributed to the presence of phytochemicals such as scopoletin, scoparone, lamonoid, tannins, saponins and steroids in Khaya senegalensis (Gbile, 1986). However, the elevated conjugated bilirubin level observed in rats administered 1000 mg kg⁻¹ b.wt. may be an indication of hepatobiliary disease.

In the present study, it was observed that administration of the polyherbal formulation at doses of 500 and 1000 mg kg⁻¹ b.wt. decreased the level of urea but had no significant change in the level of creatinine. The decreased levels of urea probably indicate that the drug did not interfere with the renal capacity to excrete this metabolite. It may also be a reflection of the preserved renal integrity of treated rats. This indicates the prevention of any significant kidney change (Thanabhorn et al., 2006). On the other hand, the normal level of serum creatinine is an indication that the test drug did not affect renal function and that renal integrity was preserved (Thanabhorn et al., 2006). This observation suggests a normal kidney function but an impaired liver function because blood urea nitrogen is affected by liver function. It is therefore possible that the product had effect on the liver rather than the kidney (Koffuor et al., 2011). However, the result of this study is in agreement with the findings of Otimenyin et al. (2009), who reported that increase in cholesterol levels and a corresponding decrease in urea level is an indication of liver damage.

The findings of the liver enzyme assay were further corroborated with histopathological studies. The histopathological examination of hepatic cells clearly revealed chronic hepatitis in treated rats. These observations suggest that the polyherbal formulations possessed significant hepatotoxic properties. On the other hand, the normal architecture observed in the histopathological examination of the testes is an indication that the polyherbal formula did not have any adverse effect on the testicular tissues. This observation is however, suggestive that the toxicity effect of CB may be organ-specific.

REFERENCES


