Evaluation of acute and sub-chronic toxicities of Venestin Cleansers: a polyherbal supplement in female Wistar Albino rats

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INTRODUCTION

Venestin cleanser (VC) is a liquid herbal remedy produced. It is composed of 60% emetin extracts (Adenopus breviflorus), 33% honey and 1.0% myrinstine extract. The therapeutic claims include elimination of toxins, cleansing of the kidney and digestive system, purification of the glands and cells. Like soybean the seeds Adenopus breviflorus is a rich source of most essential amino acids (Oshodi, 1996) and oil (Akintayo and Bayer, 2002). The health effects of honey have long been noted by humans. Honey contains powerful antioxidants with antiseptic and antibacterial properties. There has been an explosion in the use of unregulated herbal supplements due to the rising cost of western medicines and increasing demand for alternative and complementary medicine (Dara et al., 2008).

Traditional treatment involving herbal medicine is based on extensive knowledge gathered from applications of natural resources to humans; people have usually assumed that the treatment is safe. However, as use of these herbal remedies have been rapidly growing in popularity worldwide, researchers are paying as much attention to safety issues as to their therapeutic efficacy (Rousseaux and Schachter, 2003; Pinn, 2001). Many studies have therefore evaluated the safety of herbal medicines or the associated risk of adverse effects (Cho et al., 2005, MacPherson and Liu, 2005, Woodward, 2005). The use of various drugs and dietary supplements even therapeutic doses have been implicated in hepatotoxicity (Björnsson and Olsson, 2006; Durazo et al., 2004; Teschke et al., 2003). Liver toxicity from drugs, and also from alternative medicine products such as herbalist’s remedies and dietary supplements, is currently an increasingly relevant health issue (Lozano-Lanagrán et al., 2011). In Asia herbal remedies are a relevant cause of hepatotoxicity (Takikawa et al., 2009).

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Pageaux and Larrey, 2003; Stedman, 2002 have reviewed herbal remedies that have been associated with hepatotoxicity. The present study strives to complement the knowledge on the risk assessment of herbal remedies. We have investigated the acute and sub chronic toxicities of Venestin cleanser in female rats. The hepato-renal profile and effect on ovary are reported.

MATERIALS AND METHODS

Venestin cleanser® - a poly herbal formula was purchased in August 2011, from Emiola Naturalist Health Care Centre Port Harcourt Nigeria.

Animal husbandry

Female Wistar rats (120 – 180g) were used and handled as per the International Guidelines for handling experimental animals. The rats were housed in good cages in uniform lighting (12 h dark & light cycles) and acclimatized for two weeks at room temperature. The rats were fed on pellet and tap water ad libitum.

Acute toxicity test

Ten female Wistar albino rats were used for acute toxicity test according to the organization of Economic Cooperation and Development (OECD) guidelines 425 (OECD 2000 guideline). Each received a single oral-dose of 2000 mg/kg VC. The rats were kept overnight fasting prior to the administration of VC by oral gavages. After administration of the VC, food was withheld for a further 3-4 h. The rats were observed individually at least once during first 30 min after dosing, periodically during first 24 h and daily thereafter for 14 days. The following parameters were monitored: changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) (OECD, 2000).

Sub-chronic oral toxicity study

The weight matched rats were divided into three groups of 8 rats each. Group I received normal feed and water only. Rats in group II were fed with normal feed, water and 500 mg/kg VC daily. Rats in group III were fed with normal feed, water and 1000 mg/kg VC daily. In all, daily VC administration was by oral gavage. The feed and fluid intakes were measured daily and the body weights of the rats were taken weekly. Twenty four female rats were divided into three groups of 8 rats per group.

Table 1: Treatment protocol.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration</th>
<th>Number of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Feeds + water</td>
<td>28 days</td>
<td>8</td>
</tr>
<tr>
<td>500mg/kg</td>
<td>Feeds + water + 500 mg/kg of VC</td>
<td>28 days</td>
<td>8</td>
</tr>
<tr>
<td>1000mg/kg</td>
<td>Feeds + water + 1000 mg/kg of VC</td>
<td>28 days</td>
<td>8</td>
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</table>

Sample collection

Necropsy was carried out on the 28th day and animals were sacrificed under ether anesthesia. Blood sampling was by cardiac puncture. The serum was harvested for biochemical estimation. The levels of Alanine aminotransferase ALT, aspartate aminotransferase AST, alkaline phosphatase ALP, total bilirubin, conjugated bilirubin, and total protein were estimated using the Humazym MUV test kits. Electrolyte levels were estimated using the method of analysis as prescribed by Maruna (1958) and Trinder (1951).

Histopathological Examination

Specimens of liver and ovaries of both VC treated and untreated rats were fixed in 10% buffered neutral formalin for 48h. This was followed by treatment with bovine solution for 6h and processed for paraffin embedding. Sections of 5 µm thickness of the tissues (liver/ovaries) were taken separately, processed in alcohol-xylene series. The tissues were stained with alum-haematoxylin and eosin and subjected to histopathological examination.

Statistical analyses

The data are expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used for between and within group comparison. Students’ t-test was used for paired comparison. 95% level of significance (p<0.05) was used for the statistical analysis.

RESULTS

There were 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, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000mg /kg of VC. The absence of toxicity at 2000 mg/kg of venestin cleanser informed our choice of 500 and 1000 mg/kg VC. for this study. The effects of VC on feed and fluid intakes are shown in Figs. 1-2. Venestin cleanser VC (500 and 1000 mg/kg) caused increase in feed and fluid intake in dose dependent manner. An increase in the body weights of the rats was observed during the administration period of the .500 and 1000 mg/kg VC respectively, when compared with the control as shown in Fig. 3.

Fig. 1: Effects of VC on feeds intake in control and treated rat.
The effects of the VC on the following biochemical parameters Alanine aminotransferase ALT, aspartate aminotransferase AST, alkaline phosphatase ALP, total bilirubin, conjugated bilirubin, and total protein are shown in Table 2. Administration of VC caused a significant (p ≤ 0.05) increase of aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) and significant (p ≤ 0.05) reduction of Alanine aminotransferase ALT. There was a significant (p ≤ 0.05) increase in total bilirubin following administration 500 mg/kg VC. However 1000 mg/kg VC produced significant decrease of total bilirubin. There was a significant (p ≤ 0.05) increase in conjugated bilirubin and significant (p ≤ 0.05) decrease total protein after administration of 500 and 1000 mg/kg VC. The effect of VC on electrolytes level is shown in Table 3. Administration of 500 mg/kg VC led to significant (p ≤ 0.05) decrease in potassium ion level whereas 1000 mg/kg VC slightly increased the potassium ion level. VC significantly (p ≤ 0.05) decreased the chloride ion levels from 117.18±10.12 to 111.30±5.04. Bicarbonate ion levels were significantly (p ≤ 0.05) reduced in the groups that received 500 and 1000 mg/kg VC. The histology of the liver of female Wistar albino rats after administration of 500 mg/kg VC showed a widespread balloon degeneration of the hepatocytes with loss of radial arrangement of hepatocytes around the central veins. There was also perportal infiltration by chronic inflammatory cells (Fig 4b). Administration of 1000 mg/kg VC showed a mild perportal and scattered lobular infiltration by chronic inflammatory cells, widespread ballooning degeneration of hepatocytes and mild loss of radial arrangement of hepatocytes around the central veins (Fig 4c). The result of the effect of VC on histological examination of the ovary of female Wistar albino rats are shown in Fig 5(a-c). There were areas of luteinized stromal cells, normal follicle (Fig 5b). However, the results obtained from the group administered 1000 mg/kg VC showed areas of luteinized stromal cells and normal follicles. Normal fallopian tubes were also observed (Fig 5c).

Table 2: Effects of Venestin Cleanser on liver biochemical parameters of female Wistar albino rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>AST : ALT</th>
<th>Total Bilirubin (µmol/L)</th>
<th>Conjugated Bilirubin (µmol/L)</th>
<th>Total Protein (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95.17±8.38</td>
<td>103.17±16.56</td>
<td>219.05±35.70</td>
<td>1.08±1.97</td>
<td>8.50±1.28</td>
<td>6.40±0.75</td>
<td>84.0±6.57</td>
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<tr>
<td>500 mg/kg</td>
<td>73.50±10.35</td>
<td>166.83±38.36</td>
<td>300.40±106.16</td>
<td>2.27±3.71</td>
<td>9.47±5.46</td>
<td>8.28±2.96</td>
<td>76.0±10.80</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>79.00±16.42</td>
<td>187.33±23.48</td>
<td>493.07±44.63</td>
<td>2.37±1.43</td>
<td>6.75±4.36</td>
<td>8.42±6.72</td>
<td>79.7±13.65</td>
</tr>
</tbody>
</table>

n=8, Values (mean±SD). *p≤0.05 when compared with control.

Table 3: Effects of Venestin Cleanser on serum electrolyte levels of female Wistar albino rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Na⁺ (Mmol/l)</th>
<th>K⁺ (Mmol/l)</th>
<th>Cl⁻ (Mmol/l)</th>
<th>HCO₃⁻ (Mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>151.80±2.48</td>
<td>9.27±1.17</td>
<td>117.18±10.12</td>
<td>17.37±2.39</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>158.00±6.89</td>
<td>8.63±1.48</td>
<td>112.60±6.88</td>
<td>11.48±2.09</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>157.83±6.61</td>
<td>9.61±2.34</td>
<td>111.30±5.04</td>
<td>10.48±1.48</td>
</tr>
</tbody>
</table>

n=8, Values (mean±SD) *p≤0.05 when compared with control.

Fig. 4: (a) showing normal architecture of the liver of Wistar albino rats (b) showing architecture of the liver of Wistar albino rats treated with 500mg/kg of VC (c) showing architecture of the liver of Wistar albino rats administered with 1000mg/kg of VC.
DISCUSSION

Herbal supplements represent an increasingly common source of drug-induced liver injury. The study investigated the hepatorenal effects of VC - a poly herbal formula in female Wistar albino rats. The alteration in overall body weight or organ-body weight ratio is an indication of impairment in the normal functioning of the organs. Differences in organ weights arising from treatment with test substances are widely accepted parameters for the evaluation of test article-associated toxicities (UNL 2002). Since there was an increase in relative and absolute organ weight of the liver accompanied by significant change in other biochemical parameters in the treated animals at all tested doses, we may conclude that the extract is hepatotoxic (Ezeonwumelu et al., 2011; Kluwe 1981).

Assessment of liver function is very important in toxicity evaluation of drugs and herbal supplements as the liver is necessary for the survival of an organism. AST and ALT activities are commonly measured to monitor liver damage. A mild or higher activity of AST indicates liver injury or myocardial infarction (Ozer et al., 2008; Feldman and Zinkl, 2000) and the ratio of AST/ALT may be employed in disease diagnosis. An AST/ALT ratio greater than 1 suggests myocardial infarction, while a ratio less than 1 may be due to the release of ALT from the affected liver (Crook, 2006). When an AST/ALT ratio is more than 2, it is indicative of alcoholic hepatitis or cirrhosis (Sacher and Mepherson 1991). The ratio of AST/ALT in this study ranged from 2.27±3.71-2.37±1.43, an indication of the abnormal functioning of the liver. It is noteworthy that slight increases in AST and ALT are not always accompanied by significant changes of hepatic enzyme activity or imply severe liver damage (Matloff et al., 1980). The significant alterations in the indicators of liver damage (ALT, AST, alkaline phosphatase, total protein, total and conjugated bilirubin), suggests that Venestin cleanser may affect hepatocyte function in albino rat.

The elevation in the levels of alkaline phosphatase caused by 500 and 1000 mg/kg of Venestin Cleanser may be attributed to hepatic dysfunction induced by Venestin Cleanser (Arora et al., 2006).

Herbal supplements may contain a variety of chemicals that are toxic when ingested at high doses and often causes sub clinical injury to the liver which manifest only as abnormal liver enzyme test (Hiranol et al., 1992). Although the liver has the capacity to completely regenerate itself when damaged during short periods, chronic liver injury can cause a gradual progression of fibrotic or cirrhotic changes or even cancerous transformation that may result in dysfunction of the liver itself with whole-body consequences (Chen, 2005). The hepatotoxic effect of VC could be caused by a chemical contaminants or plant toxin in the herb of choice used in the production of the drug (Lee et al., 2004).

The significant increase in total bilirubin seen in the 500mg/kg VC treated and the increase in unconjugated bilirubin observed at all doses of VC in this study suggest a hepatobiliary disorder in which there is a partial or complete blockage of the flow of bile through the bile ducts, causing a buildup of conjugated bilirubin in the blood this may be as a result of the pressure on the small bile ducts caused as a side effect of the drug (Chessbrough, 2006). The reduction in the total protein concentrations following VC administration may also be as a result of liver damage or disease associated with a reduction in the production of albumin, because albumin accounts for 60% of total plasma protein and its synthesized exclusively by the liver (Chessbrough, 2006). Venestin cleanser reduced the electrolytes level (K⁺, Cl⁻, HCO₃⁻) The reduction may be due to the presence of acid, a clue to acute metabolic acidosis. When there is a presence of acid, bicarbonate level drops leading to a drop in pH value and also a drop in the buffering capacity of blood (Wilson and Zenneth, 2003). The distortion in the regulation of renal functions is responsible for the altered balance of salt and water in pathophysiological states (Mohring et al., 1975).

The increased sodium ion concentration could be that the Venestin Cleanser may have properties that affected glomerular filtrations and thereby decreasing the excretion of substances in the urine including sodium ion. Also, the increased Na+ concentration could be an indication of dehydration. (Abdulrahman et al., 2007). The decreased serum potassium ion concentration observed in 500mg/kg VC correlates well with increased sodium ion concentration. Potassium ion is a major cation of the intracellular fluid and only about 10% of the total body potassium is found extracellular. It has been observed that the intracellular potassium ion can not be measured; therefore, serum potassium concentration is not a good measure of a total body potassium because the bulk of potassium resides within the cells (Tietz et al., 2006). The decrease in potassium ion and
increase in sodium ion may suggest an adverse effect on the kidney. Abdulrahman et al. (2007) also observed similar effect on sodium and potassium ion when albino rat was administered with aqueous extract of root bark of \textit{vitis doniana}.

It is known that heightened sodium retention is usually associated and directly related with chloride ion, since most sodium ion reabsorption is linked to chloride ion reabsorption (Browse 2005).

The inverse relationship between sodium and chloride ions in this study will require further investigation. VC produced dose dependent reduction in bicarbonate ion. Normally the chloride ion is actively reabsorbed in one –for-one exchange for bicarbonate. (Guyton and Hall, 2002).

The effect of VC on chloride and bicarbonate ions suggest an alteration in the normal functioning of the kidney

Although the histology of the ovary in VC treated animals seem to suggest no harmful effects, the overall observation on the liver and kidney in this study indicate some measure of caution on the users of VC. The risk may not be negligible. In conclusion the hepato-renal picture of Venestin cleanser may be of public health importance that may necessitate an epidemiological study of users of Venestin cleanser

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Trinder, P. A rapid method for the determination of sodium in serum. Analyst. 1951; 76: (907) 596-599.

