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Toxicological evaluation of Hb cleanser® bitters on biochemical parameters in Wistar rats

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Abstract

This study was carried out to evaluate the effect of HB Cleanser bitters on biochemical parameters in male Wistar rats. Twenty-eight animals were assigned into four groups of seven animals each. Groups A, B, C received 1, 1.03 and 1.29 ml/kg of the HB bitters respectively while group D (control), received 1 ml normal saline. All administrations were done orally and daily for 28 days. The feed and fluid intake were measured daily and the body weight weekly. Phytochemical screening revealed the presence of saponins, flavonoids and cardiac glycosides. Acute toxicity results showed that the LD50 of HB Cleanser Bitters was greater than 5000 mg/kg with no mortality. There was a significant ($P<0.05$) increase in body weight. In addition, HB bitters caused at 1 ml/kg significant ($P<0.05$) increase in total bilirubin, conjugated bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and urea more than the other doses compared to negative control. A significant ($P<0.05$) decrease was observed in the levels of total protein and albumin compared to negative control. The findings from this study showed that HB cleanser Bitters may adversely affect hepatic and renal indices and therefore should be used with caution.

Keywords: HB Cleanser Bitters; Creatinine; Aspartate Aminotransferase; Albumin; Total Protein

1. Introduction

There is an upsurge in the use of medicinal plants in the treatment of diseases and infections for several decades as a primary source of healthcare. The World Health Organization estimated that about 80% of the world population use herbal medicines in the developing world [1].

Herbal medicines as defined by WHO, can be defined as phytomedicines or phytopharmaceuticals sold as over the counter (OTC) products in modern dosage forms such as tablets, capsules and liquids for oral use or dietary supplements containing herbal products, also called nutraceuticals available in modern dosage forms. These two types of herbal medicines are used by consumers in developed countries and those in urban areas of developing countries. These herbal medicines are gradually occupying increasing shelf space in modern Pharmacies. The increase in the popularity of herbal medicines may be ascribed to indiscriminate advertisement of the therapeutic claims of this product, perceived huge costs and adverse effects of orthodox drugs and the erroneous belief that herbal medicines are more effective and safer than orthodox medicines and the assertion that "it worked for my friend, neighbour or associate, so it would work for me" [2, 3].

Some individuals resort to the use of herbal medicines because of treatment failure that occurred with orthodox medicines. HB cleanser bitters is a mixture manufactured and sold in Nigeria, consisting of *Aloe vera* (25%), *Acinos*

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arvensis (20%), *Moringa oleifera* (10%), *Allium sativum* (15%), *Chenopodium murale* (15%) and *Cinnamomum aromaticum* (15%).

Some herbal preparations have been shown to exhibit some form of toxicity. Shorinwa et al,[4], reported that HB cleanser bitters caused reduction in some hematological indices, while Odangowei et al, [5], reported that prolong administration of Ruzu herbal bitters may result in renal and hepatic toxicity. Hence, it has become pertinent that the safety profile of herbal medicinal preparations should be ascertained to provide scientific information to the public so as to help them in decision making in respect of likely associated risks [6].

Therefore, this study investigated the effects of HB cleanser bitters on biochemical parameters in Wistar rats.

2. Material and methods

2.1. Animals

Adult wistar rats of both sexes purchased from the animal house of the Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria was used for the study. The animals were kept in separate plastic cages and fed ad libitum with standard feed (Broiler finisher-Guinea feeds) and had free access to water. They were maintained under standard conditions of humidity and temperature. The animals were acclimatized for two weeks before the commencement of the study. All the standard ethical requirements for experimental animals were complied with. Ethical approval was obtained from the Research ethics committee of the University of Port Harcourt.

2.2. Sub-acute toxicity study

A total of 28 male Wistar rats were randomly allotted to 5 groups. The control group was treated with normal saline while the three experimental groups were administered with 1, 1.03 ml/kg and 1.29 ml/kg of HB cleanser® bitters calculated according to the manufacturer's dosage recommendation, (representing the children dose, the adolescent dose, and adult dose respectively) for 28 days. Rats were weighed weekly; feed and fluid intake were determined daily. The animals were observed for behavioral changes during the study [4].

2.3. Collection of blood samples

At the end of the experiment, the animals were sacrificed under diethyl ether anaesthesia and their blood collected aseptically into sterile plain bottles.

2.4. Analysis of blood sample

The blood samples were sent to the University of Port Harcourt Teaching Hospital Chemical Pathology Department and each blood samples were analyzed for kidney function (Urea and creatinine) and liver function (total bilirubin, conjugated bilirubin, aspartate amino transferase, alanine aminotransferase, alkaline phosphatase, total protein and albumin) parameters.

2.5. Biochemical analysis

Alanine amino transferase (ALT) and aspartate amino transferase (AST) were assayed with Randox laboratory Kit (England) while creatinine was measured using QCA kit according to Jaffe's method which is a colometric method [7, 8]. Alkaline phosphatases were measured using the enzymatic end point method [9,10,11,12], Total bilirubin was determined with the method of Doumas et al, [13], while total protein was assayed using the burette method [23]. Urea was determined with the Diacetylmonoxime method [14]. Albumin was assayed according to the method described by [15].

2.6. Statistical analysis

All the data obtained were expressed in Mean± Standard error of Mean (SEM). Statistical analysis of data was done using one way analysis of variance (ANOVA) followed by the Students t-test using graph pad prism version 5. The statistical analysis was done to determine the significance between the control group and the treatment groups. P-values were considered statistically significant at a value of $P<0.05$.

3. Results

3.1. Effects of HB cleanser bitters on body weight.

A significant increase in the body weight was observed in all the groups that were administered with HB cleanser bitters when compared to the control in the table 1 below.

Table 1 Effects of HB cleanser bitters on body weight

Group	Dose(ml/kg)	Days				
		0	7	14	21	28
Control	5	179.70 ±2.05	188.40 ±3.51	192.00 ±3.01	183.30 ± 3.41	197.20 ± 4.01
HB cleanser	1	198.60 ±1.07*	212.00 ±3.06**	223.70 ±3.71**	222.40 ± 4.01**	233.10 ± 4.61**
HB cleanser	1.03	203.10 ±2.34*	197.90 ±4.00**	207.70 ±4.32*	219.90 ± 3.87**	208.40 ± 5.38**
HB cleanser	1.29	215.60 ±3.21*	209.50 ±2.13**	219.70 ±4.52*	224.10 ± 4.01**	225.30 ± 4.21**

Values are expressed as Mean±SEM, n=5, Significance=*P<0.05, **P<0.01

3.2. Liver function test

HB cleanser bitters produced statistically significant (P<0.05) increase in the concentrations of total bilirubin, conjugated bilirubin, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase at 1 ml/kg more than the other doses compared to the negative control. A significant (P<0.05) decrease was also observed in total protein and albumin levels when compared to the negative control as shown in table 2 below.

Table 2 Effects of HB cleanser bitters on liver function parameters of Wistar rats

Parameters	Control	HB cleanser bitters (ml/kg)		
		1	1.03	1.29
TB (µmol/L)	2.75±0.48	4.00±0.55*	3.60±0.51*	2.60±0.40*
CB (µmol/ml)	0.60±0.40	1.00±0.45	1.00±0.32*	0.20±0.20
AST (IU/L)	104.75±4.50	124.40±9.88*	125.00±8.49*	119.80±7.14*
ALT (IU/L)	16.25±4.39	36.00±3.24*	29.40±2.87*	33.60±1.83*
ALP (IU/ml)	170.50±11.93	209.40±32.95*	184.60±11.30*	164.20±14.41*
TP (g/L)	74.25±0.96	70.40±2.40*	69.80±1.28*	65.80±4.19*
ALB (g/L)	32.00±1.22	27.80±1.24*	28.00±1.79*	27.20±1.59*

Values are expressed in Mean±S.E.M, n=5, Significance= *P<0.05

3.3. Kidney function

HB cleanser bitters produced statistically significant (P<0.05) increase in the concentration of urea and creatinine when compared to the control group (Table 3).

Table 3 Effects of HB cleanser bitters on kidney function parameters of Wistar rats

Groups	Dose (ml/kg)	Urea (mmol/L)	Creatinine (µmol/L)
Control	5	7.05±0.21	61.25±1.48
HB cleanser	1	8.12±0.32**	69.60±1.68**
HB cleanser	1.03	7.40±0.29**	64.40±3.14**
HB cleanser	1.29	8.36±0.38**	66.80±3.21**

Values are expressed in Mean ±S.E.M, n=5, Significance= *p<0.05, **p<0.01, ***p<0.0001

4. Discussion

Medicinal plants are of great importance to the health of individuals and communities. Herbal preparations usage in the treatment of diseases by people is on the increase even though some of them do not have sufficient information on their safety profile. Hence, it has become expedient to evaluate the toxicity of such products in other to safeguard the health of the public [16]. Some previous studies such as that of Ogbonnia et al, [17], have shown the potential toxicity effects of herbal medicinal preparations.

The present study was aimed at the evaluation of the effects of HB cleanser bitters on biochemical parameters in rats which have close resemblance with human physiology.

A significant increase was observed in the body weights of the animals which showed that HB bitters did not adversely affect the animals.

Liver function test helps to ascertain the functionality of the liver. HB cleanser bitters showed a significant increase in total bilirubin, conjugate bilirubin, aspartate transaminase, alanine transaminase and alkaline phosphatase concentration, with decrease in total protein and albumin concentration.

Increased total bilirubin causes jaundice which implies problem in liver metabolic function because of reduced hepatocyte uptake, impaired bilirubin conjugation or reduced bilirubin secretion [18].

Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase are used as biomarkers in the estimation or evaluation of liver function or toxicity. Increase in serum levels of these enzymes indicates liver injury or toxicity [19]. AST is present in the cytoplasm of hepatocytes and other tissues, including skeletal muscle. Hepatocytes injury causes AST leakage into the extracellular compartment with subsequent increase in serum AST activity. AST activity may also be elevated when there is muscle injury [20].

HB cleanser bitters significantly increased both the AST and ALT levels which is an indication of alteration of the integrity of the liver parenchymal cells that may ultimately lead to toxicity.

Alkaline phosphatase (ALP) is an enzyme found throughout the body, but it is mostly located in the liver, bones, kidneys, and digestive system. It is a marker of hepatobiliary injury. When the liver is damaged, ALP may leak into the bloodstream High levels of ALP can indicate liver or bone disorders [21]. Alkaline phosphatase concentration may also be elevated when the bile ducts are blocked [22]. Hb cleanser bitters significantly increased alkaline phosphatase levels.

Albumin is the main constituent of serum total protein and is involved in the maintenance of plasma osmotic pressure and movement of hormones and lipids [23]. HB cleanser bitters caused a significant decrease in the albumin level. Decrease in albumin blood concentration is linked to chronic liver disease which may likely be due to the failure of the liver to synthesize enough protein especially albumin. A corresponding decrease was observed in total protein level in this study which may likely be associated with the decrease in albumin concentration.

Urea level was significantly increased in this study. Urea is a metabolic product of protein and amino acid. Urea is filtered from the blood into the urine by the glomeruli in the kidney, thus increase or decrease in urea concentration may be due to malfunctioning or damage to the kidneys. Increase in urea levels may also be linked to dehydration, high protein diet and increased protein metabolism [24].

Creatinine concentration increased at all the doses of HB cleanser bitters which was non-dose dependent. Abnormality in kidney function results in increase in creatinine concentration because of reduced blood clearance by the kidneys through glomerular filtration and by proximal tubular secretion [25].

This implies that HB Cleanser bitters may adversely affect hepatic and renal indices. This finding agrees with that of Odangowei et al, [5], which reported that Ruzu herbal bitters had adverse effects on renal and hepatic parameters in Wistar albino rats.

5. Conclusion

Hence, the findings of this study suggests that HB cleanser bitters may adversely affect hepatic and renal functions.

Compliance with ethical standards

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Disclosure of conflict of interest

All contributing authors declare no conflicts of interest.

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Statement of ethical approval

Ethical approval was obtained from the Research and Ethics Committee of the University of Port Harcourt according to international ethical requirements.

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