MULTIPLE ORGAN TOXICITY OF A NIGERIAN HERBAL SUPPLEMENT (U & D SWEET BITTER) IN MALE ALBINO RATS

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ABSTRACT

In a three month study, 3 groups of albino rats (150-200g), 539, 1077, and 1616mg/kg aqueous extract of U & Dee Sweet Bitter, were administered orally while deionized water was given to the control group. Animals had access to deionized water and were fed *ad libitum* with rat chow for 90 days. The feed and fluid consumption of the animals were measured on daily basis while the body weight was measured weekly. Animals were anaesthetized with ether after 90 days, bled sacrificed, heart, spleen and pancreas were excised and weighed. The following parameters were measured namely food and fluid intake, body weight, absolute and relative weights of the, spleen, pancreas, heart. Serum glucose, low density lipoprotein LDL, high density lipoprotein HDL and cholesterol were also determined.

Food and fluid consumption were not significantly ($p > 0.05$) affected. A significant ($p < 0.05$) increase in absolute and relative weights was observed while the weekly body weight did not show any significant difference between the control and U & Dee Sweet Bitter treated groups. A significant ($p < 0.05$) decrease in total serum cholesterol, low -density lipoprotein (LDL), and high-density lipoprotein (HDL) were seen in U & Dee Sweet Bitter treated groups. This study suggests that U & Dee Sweet Bitter may have toxic effect on the spleen, pancreas, and heart of male albino rat.

Keywords: Hypoglycemic, heart, spleen, pancreas, lipid, toxicity, U & Dee sweet bitter.

INTRODUCTION

Herbal medicines have been believed by many people to be natural and that medications of natural origin are not toxic or dangerous. There have been reports of acute and chronic intoxication resulting from the use of herbal remedies. Several researchers also reported that most herbal remedies exhibit organ specific toxicity, hence the delay in manifestation of toxic effects.

Lack of standardization is a major concern regarding use of medicinal herbal medicines (Angell and Kassierr 1998, NIEHS 1998). Herbal medicines are complex mixtures in which the active ingredient may not be known or may be only a small percent of the total product. Some are believed to achieve their beneficial effects through the combined actions of several ingredients. Little is known about chronic toxicities that might be associated with their prolonged use. There has been minimal research to assess possible systemic toxicity that might be associated with high doses or due to chronic administration of products (Miller1998, Haze *et al.,* 1999).

U & D Sweet Bitter is a registered herbal supplement prepared from roots, leaves, flowers and fruits of different herbs. It is very widely used and can be available in Eastern Nigeria. The marketing label acclaimed therapeutic indications in typhoid, malaria, and stomachache. Efforts made by regulatory authorities in ensuring safety of registered pharmaceutical products in Nigeria seem to concentrate on effects of these pharmaceuticals on renal and liver function test. The use of herbal supplements in Nigeria is high probably due to poverty and traditional beliefs. There is a dearth of information on the systemic toxicity of these herbal supplements agents especially with respect to organs other than liver and kidney.

The present study investigates if there are any adverse effects associated with chronic or high dose of U&D Sweet Bitter in the heart, spleen and pancreas of male albino rats.

MATERIALS AND METHODS

Preparation of the extract of U & D sweet bitter herbal supplement

About 5000mls of the liquid herbal supplement U&D Sweet bitter was concentrated using a vacuum evaporator for 8 hours. A total of 120g of the recovered residue was used in this study (Orisakwe *et al.*, 2007).

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Animal study

Adult male albino rats (100-150g) were supplied by the Animal Facility Centre of Department of Pharmacognosy University of Nigeria, Nsukka. Animals received water and feed (rat chow) *ad libitum* (Pfizer Pharmaceuticals Plc, Ikeja Nigeria). Twenty mature male albino rats were used in this study. These were divided into four groups of five rats each. Three of the groups were given 539, 1077, and 1616mg/kg body weight (representing 25%, 50% and 75% of the LD50 (2164mg/kg) from a previous study (Obi 2006) of the aqueous extract (p.o), respectively while the control group received deionized water only for 90 days. Food and fluid intake were monitored daily while body weight was measured weekly. Animals were anaesthetized with ether after 90 days of dosing, bled and sacrificed; heart, spleen and pancreas were excised and weighed. The parameters measured include food and fluid intake, body weight, absolute and relative weights of the, spleen, pancreas and heart.

Blood for chemical analysis was collected by retro-orbital venopuncture and serum total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) was determined. Serum cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined by enzymatic method and estimated colorimetrically by the method outlined by Trinder, (1996). About 2ml of whole blood were collected in a fluoride anticougulated sample container and centrifuged at 3500rpm for 5 minute and the plasma separated and analyzed for glucose using the method of Richardson (1977).

STATISTICAL ANALYSIS

Values were reported as mean $\pm$ SEM. Analysis of variance (ANOVA) was employed for between and within group comparison while student's t-test was used for paired comparison. All differences were considered significant at 5% level, therefore a p-value of $p \leq 0.05$ was considered statistical significant.

RESULTS

Table 1 shows the feed and fluid intake, the final body weights, liver weight (relative and absolute) of albino rats treated with the extract of U&D Sweet Bitter herbal drug compared with the control. The result revealed that there was non-significant increase ($p > 0.05$) in food and fluid intake in all the treated animals as compared to the control. There was no significant difference in the food and fluid consumption of the rats. There was gradual increase in body weight. There was a dose dependent significant ($p \leq 0.05$) changes in the absolute weights of the heart, spleen and pancreas compared to the control.

Table 2 shows the effect of U & D Sweet Bitter on the lipid profiles and serum glucose levels. From the table, administration of 539, 1077 and 1616mg/kg of the extract caused a significant ($p<0.05$) decrease in serum cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) compared to the control. Administration of U&D Sweet Bitter tended to increase serum glucose level.

DISCUSSION

We have investigated the chronic effects of U&D Sweet Bitter in simulated toxic doses which represent the actual life situation in which the general public take these herbal supplements using the animal model. Our results show that U & D Sweet Bitter caused significant ($p < 0.05$) decrease in total serum cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). U & D Sweet bitter did not seem to have any beneficial effect in the reduction of plasma glucose level a feature which would have been desirable in the management of diabetes mellitus as claimed by the herbal practitioners. The effect of U&D Sweet Bitter on the lipid profile suggests a possible cardio toxic effect. Kluwe 1981 and Simmoni et al 1995 stated that any change in the organ weight (both relative and/or absolute) coupled with a change in one of the biochemical parameters is an indication of the toxicity of the test substance. U&D Sweet Bitter administration significantly decreased the absolute and relative weight of the heart in this study.

Chemical compounds in herb can change heart rate, blood pressure and glucose levels. Hence people with cardiac problems, high blood pressure, or blood sugar disorder such as diabetes must be especially cautious. U & Dee Sweet Bitter is a mixture of roots, leaves, flowers and seeds of different herbs and these make for potential misidentification or cross contamination of the active ingredients. Among the phytoconstituents found in U & Dee Sweet Bitter extract are flavonoids, steroids that may be mainly responsible for its pharmacological activities (Daffallah and a-Mustafa, 1996). Cholesterol level was decreased significantly ($p < 0.05$) in U & Dee Sweet Bitter treated groups. Previous study revealed a decreased cholesterol level in rats (Onyenekwe *et al*, 1999; Hussaini *et al*, 2004). Flavonoids inhibit cyclo-oxygenase and 5-lipooxygenase, which have been postulated to be involved in cardiovascular disease (Laughton *et al.*, 1991).Unlike the flavonoids found in red wine which tends to be cardio protective the flavonoids found in U & Dee Sweet Bitter did not show this mechanism as evidenced by the lipid profile and the absolute and relative weight of the heart.

Although herbal supplements may be considered to be safe, some are known to be toxic at high doses and others may have potentially adverse effect after prolonged use. Because these are not prescribed by the physician or dispensed by pharmacist report of their adverse effect are largely inaccurate. The general public is largely unaware

Table 1: Mean food intake, fluid consumption, absolute and relative weight of pancreas of rats exposed to aqueous extract of U & D Sweet Bitter. N=5; Relative weight = absolute weight/ relative weight x 100; *significantly increased compared to control.

Dose (mg/kg)	Feed (g)	Fluid (l)	Pancreas	Spleen	Heart	Final body wt (g)
Control	118.15 + 27.98	172.99 + 32.39	0.31 + 0.09 (0.72 + 0.04)	0.28 +0.02 (0.64 + 0.02)	0.35 + 0.02 (0.80 + 0.04)	231.56±9..32
539	119.90 + 32.79	183.74 + 46.83	0.39 + 0.04 (0.86 +0.10)	0.24 + 0.01 (0.52 + 0.04*)	0.46 + 0.02* (0.98 +0.05)*	211.68 ±3.08
1077	122.08 + 25.96	182.77 + 52.35	0.66 + 0.08* (1.58+ 0.6.52*)	0.45 + 6.52* (1.08 + 0.12)	0.47 + 0.04* (1.16 + 0.12)*	242.98± 8.19
1616	120.98 + 30.55	180.81 + 52.35	0.44 + 0.03* (0.8 + 0.5.83*)	0.40 + 2.80 (0.80 + 0.07)	0.61 + 0.07* (1.26 + 0.12)*	202.08± 5.64

Table 2: Lipid profiles and serum glucose level in U&D Sweet Bitter of the treated groups and control

Dose (mg/kg)	Cholesterol(mg/dl)	LDL(mg/dl)	HDL (mg/dl)	Glucose level (mmol/L)
0.00[a]	101.60 ± 0.93	37.08 ± 3.30	46.92 ± 3.09	4.14± 0.09
539	78.60 ± 3.94*	11.78 ± 0.68*	14.56 ±1.10*	6.16 ± 0.13
1077	66.80 ± 2.15*	6.82 ± 0.14*	9.30 ± 0.76*	5.58 ± 0.15
1616	57.60 ± 2.22*	5.04 ± 0.56*	6.76 ± 0.41*	4.68 ± 011

Values are expressed as Mean ± SEM for n = 5, * Significantly different from control $p < 0.05$, a = Deionized water

that adverse health effects can be associated with the use of herbal supplements. These public health issues have been attributed to many factors ranging from overdosing, contaminated formulations to the inherent toxicity of the herbs of choice (Hazel *et al.,* 1999).

Taken together the present work has provided a basis to widen the scope of the required and useful toxicity tests to be carried before herbal supplements can be registered. In all our results suggest that U&D Sweet Bitter may have systemic adverse effects i.e. extra hepatic and extra renal effects namely on the heart, spleen and the heart of the male albino rat. We suggest further investigations of the observations made in this study at cellular the cellular level.

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