

Full Length Research Paper

Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes

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The present study was carried out to evaluate acute and subacute toxicity of a hydroalcoholic extract of a mixture (1:1) of *A. congensis* bark and *X. aethiopica* fruits used locally in the treatment of diabetes. Acute toxicity of the extract was evaluated in Swiss albino mice. The animals were fed with the hydroalcoholic extract between the doses of 1.0 to 20.0 g/kg body weight and were observed continuously for the first 4 h and for every hour for the next 24 h, then 6 hourly for 48 h. Wistar rats were also fed with different doses of the extract for 30 days and effects on biochemical parameters evaluated (subacute toxicity model). The LD₅₀ of the extract was found to be above 20.0 g/Kg body weight. There was reduction in the plasma glucose and low-density lipoprotein (LDL)-cholesterol levels, and increase in high-density lipoprotein (HDL)-cholesterol level in the treated animals. A significant increase in the body weight was observed for groups treated with lower doses of the extract while groups treat with higher doses showed no significant weight increase. Aspartate aminotransferases (AST) and alanine aminotransferases (ALT) levels were not affected at lower doses of the extract but there was increase in creatinine levels in all the treated animals. The extract demonstrated good hypoglycaemic effects by lowering the plasma sugar level and also had some beneficial and reduction effects on cardiovascular risk factors. There was no evidence of drug-induced symptoms or death at all the doses of the extract administered in acute study but subacute results revealed a tendency to cause kidney problems on a long-term use.

Key words: Acute, subacute, toxicity, *Alstonia congensis* and *Xylopia aethiopica*, diabetes.

INTRODUCTION

Plant-based medicaments had served from the outset as the most important therapeutic weapon available to man to fight various human and animal diseases. The exclusive use of herbal remedies to treat and manage ailments continued until the introduction of modern synthetic medicines. The advent of synthetic medicines in the health care system coupled with industrialization, urbanization in the most developed countries, and embracing of western

culture by the developing countries, made the use of herbal products to decline from about the beginning of the 20th century up to the 1970s (Wills et al., 2000). However, in recent times there is a renewal and growing interest in the use of plant-derived biologically active compounds as drugs or as leads in the manufacture of more potent medicaments (Houghton and Raman, 1998). Plants, therefore, remain the main source of the active drugs from a natural source and are still indispensable in the traditional medicine for treating a number of diseases. Traditional medicines are used by about 60% of the world population both in the developing countries and develop-

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ed countries where modern medicines are predominantly used (Mythilypriya et al., 2007).

In Nigeria as in other developing countries, traditional medicine accounts for more than 80% of rural populace health needs, with the practitioners formulating and dispensing the recipes. The medicaments are prepared most often from a combination of two or more plant products which many a time may contain active constituents with multiple physiological activities and could be used in treating various disease conditions (Pieme et al., 2006). They are administered in most disease conditions over a long period of time without a proper dosage monitoring and consideration of toxic effects that might result from such a prolonged use. The warning regarding the potential toxicity of these therapies means that the practitioners should be kept abreast of the reported incidence of renal and hepatic toxicity associated with the ingestion of medicinal herbs (Tédong et al., 2007).

A herbal formulation prepared with *Alstonia congensis* bark and *Xylopiya aethiopica* fruits in equal proportion are one of the popular local herbal preparations employed in the treatment of diabetes and is consumed over a long period of time in the course of treatment. These plants have demonstrated a wide spectrum of biological activities and have played a crucial role in traditional medicine because of their valuable physiological and pharmacological properties.

A. congensis contains various alkaloids (Evans, 1989). Its different parts are of medicinal value in various regions or countries in Africa (Burkhill, 1985), while *X. aethiopica* commonly known as negro pepper fruits, and locally called "Uda" by the Igbos of Nigeria is a highly valued medicinal plant. Among the Igbos the fruits are used as spices and the aqueous decoction is used especially after childbirth probably for its antiseptic properties and to arrest bleeding. The fruits have been found to contain volatile aromatic oil, fixed oil and rutin (Burhill, 1985). The fruits preparations are also employed locally to enhance good health and promote longevity. It has been reported to act as antioxidant, hypolipidemic and hypoglycaemic agent thereby testifying to its use as an antidiabetic agent. It is also used in the treatment of bronchitis, stomach-aches, dysenteric conditions, febrile pains and rheumatism (Ameyaw and Owusu-Ansah, 1998).

The preliminary phytochemical analysis of the extract indicated positive results for alkaloids, polyphenols and terpenoids. For a plant or herbal preparation containing active organic principles to be identified for use in the traditional medicine, a systemic approach is required for the evaluation of efficacy and safety through experiment and clinical findings (Mythilypriya et al., 2007).

The aim of this study was to evaluate the safety of the *A. congensis* bark and *X. aethiopica* fruits mixtures extract by carrying out toxicity studies in animals. Acute and subacute toxicity evaluations are required to establish potential adverse effects of this valuable herbal

preparation.

MATERIALS AND METHODS

Plant materials

The plant materials, *A. congensis* bark and *X. aethiopica* fruits were bought from Mushin market in Lagos suburban and were authenticated by Prof. Dele Olowokudejo of the Department of Botany and Microbiology, University of Lagos, Lagos. The voucher specimens LUH 2054 and LUH 2079 respectively were deposited at the Department of Botany Herbarium. The plant materials were dried at an ambient temperature between 35 – 45°C in an oven for five days and powdered to coarse particles. 700 g of the powder containing an equal amount of each plant materials was macerated with 2.5 L ethanol (80%) for seven days with frequent stirring. After filtration, the solvent was removed under reduced pressure in a rotary evaporator at a temperature below 50°C and dried to a constant weight of 32.45 g (4.6% yields). For each dose used, the volume administered was calculated using Tédong et al. (2007) equation as follows:

$$V \text{ (ml)} = (D \times P) / C$$

Where D = dose used (g/kg body weight), P = body weight (g), C = concentration (g/ml), and V = volume.

Acute toxicity test

Thirty five (35) male and female Swiss albino mice weighing 20 – 25 g were used for the acute toxicity study. They were randomly distributed into one control group and six treated groups, containing five animals per group and were maintained on standard animal diet (Feeds Nigeria Ltd) and provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. After fasting the animals over-night, the control group received 0.3 ml of 2% Tween 80 solutions orally. Solution of the extract was prepared by dispersing 8.0 g of the dried extract with 10 ml of 2% tween 80 solution and each treated group received the extract doses as follows: 1.0, 2.50, 5.0, 10.0, 15.0 and 20.0 g/kg orally. The animals were observed continuously for the first 4 h and for every hour for the next 24 h, then 6 hourly for 48 h after administering the extract to observe any changes in general behavior or other physiological activities (Shah et al., 1997; Bnrger et al., 2005)

Subacute test

Male and female Wistar albino rats weighing 160 ± 10 g were used. They were allowed to acclimatize to the laboratory conditions for seven days and were maintained on standard animal feeds and provided with water *ad libitum*. The animals were weighed and divided into five groups of five animals each. After fasting the rats overnight, the control group received a dose of 0.5 ml of 2% Tween 80 solution orally once a day for 30 days. The four treated groups respectively received the following doses: 50, 100, 250 and 500 mg/kg of the hydroalcoholic extract dispersed with 2% Tween 80 solutions orally once a day for 30 days (Pieme et al., 2006; Joshi et al. 2007; Mythilypriya et al., 2007). The animals were then weighed every four days, from the start of the treatment, to note weight variation. At the end of the experiment, they were anaesthetized with warm urethane and chloralose (25%: 1%v/v) at a dose of 5 ml/kg body weight and blood collected via cardiac puncture in two tubes: one with EDTA for immediate analysis of haematological

parameters and to separate plasma for biochemical estimations. The collected blood was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which was analyzed for total cholesterol, total triglyceride, HDL-cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan et al., 2001). LDL-cholesterol levels were calculated using Friedwald equation (Crook, 2006). Plasma was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine by standard enzymatic assay methods (Sushruta et al., 2006). Plasma glucose contents and protein contents were determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002). Haematocrit was estimated using the methods of Ekaidem et al. (2006). Haematocrit tubes were filled by capillary action to the mark with whole blood and the bottom of the tubes sealed with plasticide and centrifuge for 4 - 5 min using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along a "critocap" chart until the meniscus of the plasma intersects the 100% line. Haemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method (Ekaidem et al., 2006).

Phytochemical evaluation of the crude extracts

Phytochemical screening of the extract for the presence of secondary metabolites was performed using the following reagents and chemicals: alkaloids with Mayer reagent and Dragendorff's reagent (Farnsworth, 1966; Harborne, 1998), flavonoids with the use of Mg and HCl (Silva et al., 1998; Houghton and Raman, 1998); tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds (Houghton and Raman, 1998).

Statistical analysis

All data collected were summarized as mean \pm SEM. Significant differences were determined using a Student's t- test and the differences were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

There is, in recent times, a growing and increasing interest in herbal medicines. Consequently herbal medicines have received greater attention as an alternative to clinical therapy leading to increasing demand (Mythilypriya et al., 2007). The exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is very common in some rural Nigerian communities. Experimental screening method is, therefore, important in order to ascertain the safety and efficacy of herbal products as well as to establish the active component of these herbal remedies.

In the acute toxicity study of the extract, no changes in the behavior and in the sensory nervous system responses were observed. Also no adverse gastrointestinal effects were observed in male and female mice used in the experiment. All the mice that received 20.0 g/Kg dose of the extract survived beyond the 24 h of observation. The median acute toxicity value (LD_{50}) of the extract must be above 20.0 g/Kg body weight. According to Ghosh (1984) and Klassen et al. (1995) the extract can be classified as non-toxic, since the LD_{50} was found to be more than 15.0 g/Kg.

There was a significant increase ($p < 0.05$) in the body weights of the animals treated with the lower doses of the extract (50 and 100 mg/kg) compared with the control while at higher doses (250 and 500 mg/kg) there was neither decrease nor a significant increase in the weights of the animals (Table 1). The fairly constant body weight of the animals treated with higher doses of the extract might be as a result of suppression of fats accumulation and deposition in the organs and muscles of the animals. The effects of the extract on the organs are summarized in Table 2. The macroscopic examinations of the organs of the animals treated with various doses of the extract did not show any changes in colour compared with the control. Also, there were no significant changes in the organs weight of the treated animals compared with the control. Since no death was recorded in the acute toxicity study, and no changes in animal behavior and in organs weight were observed at all doses used, the extract or its herbal formulation can be claimed to be non-toxic.

The effects of the extract on the biochemical parameters are summarized in Table 3. The significant decrease ($p < 0.05$) in the plasma glucose level especially at higher doses in the treated rats compared with control might be due to the presence of hypoglycemic components in the extract. This observation gives credence to the use of the herbal product as a hypoglycemic agent.

There was no significant change observed in the protein levels of the rats treated with lower doses of the extract (50 and 100 mg/kg) compared with control, while an observed significant decrease in the protein levels of the rats treated with a high dose (500 mg/kg) may be a sign of impaired renal function. Also, there was a significant increase ($p < 0.05$) in the plasma creatinine levels of all the treated groups. The elevation in the plasma creatinine concentration indirectly suggests kidney damage specifically renal filtration mechanism (Wasan et al., 2001).

The liver releases alanine aminotransferase (ALT) and an elevation in plasma concentration are an indicator of liver damage. The liver and heart release AST and ALT, and an elevation in plasma concentration are an indicator of liver and heart damage (Wasan et al., 2001; Crook, 2006). There was no significant increase in AST and ALT in the animals treated with lower doses of the extract compared with control but a significant increase in ALT was observed in the group treated with a high dose of the extract (500 mg/kg). This implies that the extract at the doses used had no effects on the heart tissue but at a high dose could have some deleterious effects on the liver tissue.

The observed decrease in plasma total cholesterol (TC) level might be due to the presence of hypolipidemic agents in the extract while the increase in the triglyceride (TG) levels could be secondary to a variety of disorders that might be induced by the extract (Ellefson and Caraway, 1982). A significant increase in HDL-cholesterol levels in all the treated animals and reduction

Table 1. The effects of the extract on weight changes in control and treated rats of subacute toxicity studies.

Extract	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28	Day 30
Control	160.01± 2.7	162.03±2.18*	164.06±1.80	165.51±1.10	167.60±2.5	168.75± 2.7	168.75± 2.2	170.05±2.0	170.72 ±2.70
50 mg/kg	150.01± 0.1	150.20± 0.4*	151.3± 1.2*	151.6±1.82	152.51± 0.20	154.50 ± 0.2	155.3 ± 0.3	156.3 ± 6.3	160.02± 0.01
100 mg/kg	150.02 ± 0.12	152.40±2.80	152.7±0.79**	153.04±1.4	155.41±1.30	160.25 ± 2.36	160.20 ± 2.3	165.06± 2.01	165.50 ±3.18
250 mg/kg	150.01±0.30	150.20± 1.50*	148.02± 7.5**	148.20±6.32	153.75±4.97	148.61±11.2	150.04± 1.12*	150.50± 0.36	151.02± 2.01
500 mg/kg	160.20± 6.12	161.25± 6.92**	155.10± 4.8	153.75±4.97	153.75±4.97	163.05± 2.25*	163.5±1.2	163.7± 3.21	163.83± 5.21

Mean ± SEM (n = 5).

*p < 0.05; ** p < 0.01 vs control group.

Control group received 0.5 mL 2% Tween 80 solution.

Table 2. The effects of the extract on kidney, heart, liver and brain in control and the treated groups.

Organ	Control	50 mg/kg	100 mg/kg	250 mg/kg	500 mg/kg
Heart (g)	0.49 ± 0.03	0.41 ± 0.08*	0.49±0.02**	0.49 ±0.01**	0.45±0.06
Kidney (g)	0.93 ± 0.10	0.77± 0.18*	0.96±0.051**	0.933 ±0.06	0.85±0.07
Liver (g)	0.85 ± 0.07	4.22± 0.53	5.14±0.57	5.10 ±0.63*	4.70±0.43**
Brain (g)	1.17± 0.14	0.97±0.08*	1.07 ±0.081*	1.17±0.04	1.07±0.08*

Mean ± SEM (n = 5).

*p < 0.05; ** p < 0.01 vs control group.

Control group received 0.5 mL 2% Tween 80 solution.

Table 3. Effect of daily administration of the extract for 30 days on biochemical profiles of control and treated rats

Parameter	Control	50 mg/kg	100 mg/kg	250 mg/kg	500 mg/kg
Glucose (mg/dl)	103.96 ± 0.2	90.85 ± 0.12	81.18±0.32	58.83± 0.52	52.03 ± 1.50
Cholesterol (mg/dl)	58.46 ± 0.60	45.00± 0.03**	62.58±0.42	42.58 ± 1.4	19.35 ± 0.7
Triglyceride (mg/dl)	56.87± 0.45	66.69± 1.50	83.29± 0.02	118.37± 0.02	103.31± 0.04
HDL (mg/dl)	95.31±0.002	104.5±0.007	163.67±0.07	150.39±0.49	140.03±0.39
LDL (mg/dl)	81.44 ± 2,50	32.48± 4.75	95.60± 0.60	71.77± 3.50	86.70± 0.50*
Protein (g/dl)	6.89 ± 0.32	7.75 ± 0.25	7.26 ±0.60	5.90 ± 0.20	3.31±0.15
Creatinine (mg/dl)	0.15 ± 0.003	0.17± .001*	0.22 ± 0.05	0.50 ± 0.01	0.52 ± 0.001
AST IU/L	57.18 ± 1.20	55.69 ±2.50**	53.14± 0.02	65.48 ± 2.3	62.83 ± 0.60
ALT IU/L	24.54 ± 1.80	28.15 ± 2.52**	2219.79±2.6	34.72 ± 3.6	55.85± 2.40

Mean ± SEM (n = 5).

*p < 0.05; ** p < 0.01 vs control group.

Control group received 0.5 mL 2% Tween 80 solution.

Table 4. Effect of the extract on Haematological parameters of the control and treated animals.

Parameter	Control	50 mg/kg	100 mg/kg	250 mg/kg	500 mg/kg
Haemoglobin (mMol/L)	10.60 ±0.3	10.80 ± 0.25**	11.60± 0.40	11.30± 0.40	11.60± 0.40
PCV %	45.80± 2.20	46.20±2.3*	46.85± 1.52	44.85± 2.50	45.55± 0.50**

Mean ± SEM (n = 5).

*p < 0.05; ** p < 0.01 vs control group.

Control group received 0.5 mL 2% Tween 80 solution.

in LDL-cholesterol levels in some treated animals were observed. This showed that the extract had some beneficial effects by reducing cardiovascular risk factors, which contribute to death of diabetic patient (Barnett and O'Gara, 2003), and establishes the use of the formulation as a hypoglycaemic agent.

Phytochemical screening (result not tabulated) indicated the presence of alkaloids and polyphenols. Polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits, which include lowering of blood lipids. Furthermore recent reports have suggested that several plant sterols reduce serum cholesterol by the inhibition of intestinal cholesterol absorption (Sushruta et al., 2006). Thus, it can be suggested that the synergistic interaction of polyphenols and tannins contents in the extract may impart hypolipidemic property to the herbal preparation.

There were no significant changes observed in the hemoglobin contents and in the packed cell volume (PCV) of the treated animals compared with the control (p > 0.01) (Table 4). The extract did neither improve nor produced any deleterious effects on the hematological parameters. The little increase in the hemoglobin levels might be due to the increased absorption of iron. The increase might be speculated to be due to the immunopotentiating effect of *X. aethiopica* (Ameyaw and Owusu-Ansah, 1998).

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