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RESEARCH PAPER

PRECLINICAL TOXICITY STUDY OF THE PHYTOMEDICINE - BEE HONEY AND *MUSA PARADISIACA* EXTRACT- IN RODENTS

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ABSTRACT

This study was designed to evaluate the safety of the phytomedicine – bee honey and *M. paradisiaca* drug, through acute and subchronic toxicity studies in rodents. Acute toxicity of the phytomedicine was evaluated in Swiss albino mice using graded oral-doses of the drug in the range of 1.0 to 20.0 g/kg b.wt orally and observed continuously; first for 4hrs, hourly for the next 24hrs and then 6-hourly for 48hrs. Subchronic toxicity was investigated with different concentrations of the drug for 30 days and the effects on biochemical and hematological parameters evaluated. The median acute toxicity value (LD_{50}) of the phytomedicine was 18.840g/kg b.wt. The drug significantly reduced ($p<0.05$) plasma glucose and low density lipoprotein levels, but increased high density lipoprotein in the treated groups compared to the control. Aspartate aminotransferases and creatinine levels were significantly increased especially in the group treated with highest dose of the drug while significant decrease in alanine aminotransferases level was observed. The high LD_{50} value of the drug implies the drug could be safe for use. The study revealed that the drug had good reducing effects on hypoglycemia and the cardiovascular risk factors but that long term use can cause kidney problems.

Keywords: Acute toxicity, Sub-chronic toxicity, Musa paradisiaca, Bee honey

INTRODUCTION

The recent revolution and growing interests in the use of plant based medications in the treatment of a variety of diseases have led to the increase in the demands of herbal preparations. The exclusive use of herbal drugs in the management of certain ailments is now common in most Nigerian rural communities and other developing communities of the world. 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 (Ogbonnia et al., 2008).

Herbal recipes are most often prepared from a combination of two or more plant products with the belief that the more the number of plant products included, the more effective is the preparation in the concurrent treatment of various disease conditions (Pieme et al., 2006; Tédong et al., 2007). A single herbal recipe, therefore, may have many traditional claims as a result of various organic constituents present in the preparation. The preparations may be administered in certain disease conditions over a long period of time without a proper dosage monitoring and consideration of toxic effects that might result from such prolonged use. Warning about the potential toxicity of such herbal therapies demands 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).

Musa paradisiaca called *"Ojoko"* by the Igbos in the Eastern Nigeria is a valuable economic plant and a cherished medicinal plant in the area. Different parts of the plant are widely used by the traditional herbalists. Remedies prepared with the roots alone or in combination with other herbs are used locally in the treatment of diabetes and other diseases. Bee honey is a sweet yellow to rich amber colored viscous fluid that has been recognized for its food and medicinal values (Bogdanov et al., 2008). It has been evaluated for antibiotic effects in wound healing (Fakoor and Pipelzadeh, 2007; Jeffrey et al., 1996). Also chemical analyses showed that honey possess surprising quantities of antioxidants, non-nutritive agents that can retard biologically destructive chemical reactions that cause rancidity in food (Llesuy et al., 2001; Gomez et al., 1998). Combination therapy is more common because traditional medical practitioners believe in the synergistic effect of these mixtures over preparations from a single plant product.

The aim of this study was to evaluate the safety of the phytomedicine -bee honey and *M. paradisiaca* by carrying out the acute and subacute toxicity studies in the rodents. Subchronic toxicity evaluation is required to establish potential adverse effects of this valuable medicinal plant.

MATERIALS AND METHODS

The phytomedicine: Bee honey and *M. paradisiaca* mixtures - was ceded by a traditional practitioner, Agu Titus Iwuala Clinic, of No 105 Mushin- Itire Road, Itire, Lagos, Nigeria

Composition of the phytomedicine:

M. paradisiata dry extract	22.5g
Bee Honey	20ml
Purified water (qs)	100ml

The phytomedicine is a clear and slightly thick brown liquid in amber glass bottle of 600ml.The prescribed dose for human is equivalent to three table spoon (30ml) three times a day. This was filtered through muslin cloth and 300ml freeze dried to give a gel (47.52g).

Animals: Swiss mice (20 - 25g) and Wistar rats (160 ± 20g) of either sex obtained from the Laboratory animal Center, College of Medicine, University of Lagos, Idi-Araba were kept under standard environmental condition of 12/12hr light/dark cycle. They were housed in cages (5 animals per cage), and were maintained on animal cubes (Feeds Nigeria Ltd), provided with water *ad libitum.* They were allowed to acclimatize for seven days to the laboratory conditions before the experiment.

Acute toxicity study: The toxicity study was carried out using thirty- five (35) male and female Swiss albino mice. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. After allowing the animals to fast overnight, the control group received 0.3ml of Tween 80 (2%) solution orally. The doses 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0g /kg were respectively administered orally to the groups from a 80% (w/v) solution of the gel prepared by dispersing 16g of the gel with 7ml Tween 80 (2%) solution in a 100ml beaker and transferred to a 20ml volumetric flask. The beaker was thoroughly rinsed with the Tween solution and added to the volumetric flask and the volume made to mark with the Tween solution. The animals were observed continuously for the first 4 hours and then for each hour the next 12 hours and at 6 hourly interval for the next 56 hours after administering of the drug to observe any death or changes in general behavior and other physiological activities (Bürger et al., 2005; Shah et al., 1997).

Subchronic test: Male and female Wistar rats weighing 160g ± 10g 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: 100, 250, 500 and 750mg/kg of the gel orally once a day for 30 days (Joshi et al., 2007; Mythilypriya et al., 2007; Pieme et al., 2006). The gel suspension (12 %w/v) was prepared by dispersing the gel (12g) with 45ml of Tween 80 (2%) solution in a beaker, and transferred to a 100ml volumetric flask. Then the beaker was rinsed with the solution and transferred to the volumetric flask and volume made to mark with the Tween solution.

Sample collection: The animals were weighed every five days, from the start of the treatment, to note any weight variation. At the end of the experiment the animals were starved overnight and on the31st day they were anaesthetized with warm urethane and chloralose (25%:1%v/v) at a dose of 5ml/ kg and blood collected via cardiac

puncture in two tubes: one with EDTA for analysis of haematological parameters and the blood chemistry and the other with heparin to separate plasma for biochemical estimations.

Sample analysis: The heparinized blood was centrifuged within 5 min of collection at 4000g for 10min to obtain plasma, which was analyzed for total cholesterol, total triglyceride, and HDL-cholesterol levels by 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 according to 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 minutes 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).

Statistical analysis: Analysis of Data was done using Statistical Package for Social Sciences version 16.0. One way analysis of variance and t-test were used to compare means. Means ± SEM are shown in all tables. Level of significance was set at $p<0.05$ or $p<0.01$.

RESULTS

The acute toxicity study (Table I) recorded 60% death for all the animals that received 20.0g/kg b.wt of the extract and 20% for animals that received 15.0g , while there was no death in the animals that received 10.0g/kg b.wt and less. The median acute toxicity (LD_{50}) of the aqueous ethanolic extract of the seeds was determined to be 18.840g/kg body weight.

The effect of the extract on the body weights of the control and treated animals is shown in Table II. Generally, there was significant increase ($p<0.05$) in the body weights of the treated animals compared with the control. A drop in the weight of the control was observed in the day 10 and a dramatic increase was witnessed thereafter to the end of the experiment. The mean percentage increase in the weights of all the treated animals compared with the control was significant and is shown in Figure I.

The results of the effect of the extract on the organs are presented in Table III. Significant changes ($p < 0.01$) in the weights of various organs were observed only in the animals treated with the highest doses of the extract (500 and 750mg/kg b.wt respectively). Macroscopic examinations did not show any changes in colour of the organs of the treated animals compared with the control.

Table IV is a summary of the results of the effects of drug on the biochemical parameters. There was significant decrease ($p < 0.05$) in the plasma glucose level especially at the highest dose treated rats compared with control. A significant increase ($p < 0.05$) in the plasma protein levels were observed in the 500mg/kg and 750mg/kg groups compared with control. There was also significant increase in creatinine level compared to control. There was a significant increase in AST level while a significant decrease in ALT level was observed in all treated animals. Also significant decreases ($p < 0.05$) in the plasma total cholesterol (TC) was observed in the group that received the highest dose of phytomedicine while decrease in triglyceride (TG) and LDL-cholesterol levels and significant increase ($p < 0.05$) in HDL-cholesterol levels were observed in all the treated animals compared with the control. .

The effects of the drug on the blood components and the electrolytes are presented in Table V. No significant changes ($p< 0.05$) were observed in the hemoglobin contents, packed cell volume (PCV), red blood cells (RBC), and white blood cells (WBC) contents in the groups treated with extract compared with the control. There was also no significant increase or decrease in the calcium or phosphorus levels compared to the control.

DISCUSSION

Herbal medicines in recent times have received greater attention as alternative to orthodox clinical therapy; leading to subsequent increase in their demand (Hussain and Eshrat, 2002). In rural Nigerian communities, the exclusive use of herbal drugs, prepared and dispensed by unscientifically trained herbalists, for treatment of diseases is still very common. To this regards, Ogbonnia et al. (2008) reported that experimental screening method is, therefore,

important for ascertaining the safety and efficacy of herbal products as well as to establish the active components of these herbal remedies.

Table I: The acute toxicity of the phytomedicine-bee honey and *M.paradisiaca* in mice

Group Mice	No of	Doses of extract (g/kg)	Number of dead mice	%Cumulative of dead mice
1	5	control	0	0.0
2	5	1.0	0	0.0
3	5	2.0	0	0.0
4	5	5.0	0	0.0
5	5	10.0	0	0.0
6	5	15.0	1	25.0
7	5	20.0	3	100.00

Control group: each mouse received orally 0.3mL Tween80 (2%) solution

Table II: The effects of the drug on weight changes in the control and treated rats in the subchronic toxicity study

Dose	DAY1	DAY 5	DAY10	DAY15	DAY 20	DAY 25	DAY 30
Control	170.01± 2.70	171.03±2.18*	169.06±1.80	73.51±1.10	175.60±2.50	178.75±2.70	179.65±2.20
100mg/kg	165.01±2.10	172.20± 0.4*	174.30±1.20*	180.06±1.82*	181.51± 0.20*	184.50 ± 0.2*	186.30±0.3*
250mg/kg	152.03±0.30	156.20±1.50*	160.02±7.50**	163.20±6.32	168.75±4.97*	170..61± 1.2*	175.04± 1.5*
500mg/kg	160.12±4.20	161.25±6.92**	163.10±4.80	167.75± 4.97	168.75±4.97	170.05±2.25*	173.5± 1.2*
750mg/kg	154.20±6.12	157.25±6.92*	162.10±3.80	165.75±4.97	168.75±4.97	170.05±2.25*	172.5± 3.0*

Mean ± SEM, (n=5) *p<0.05; ** p<0.01 vs. control group. Control group received 0.5mL Tween 80(2%) solution

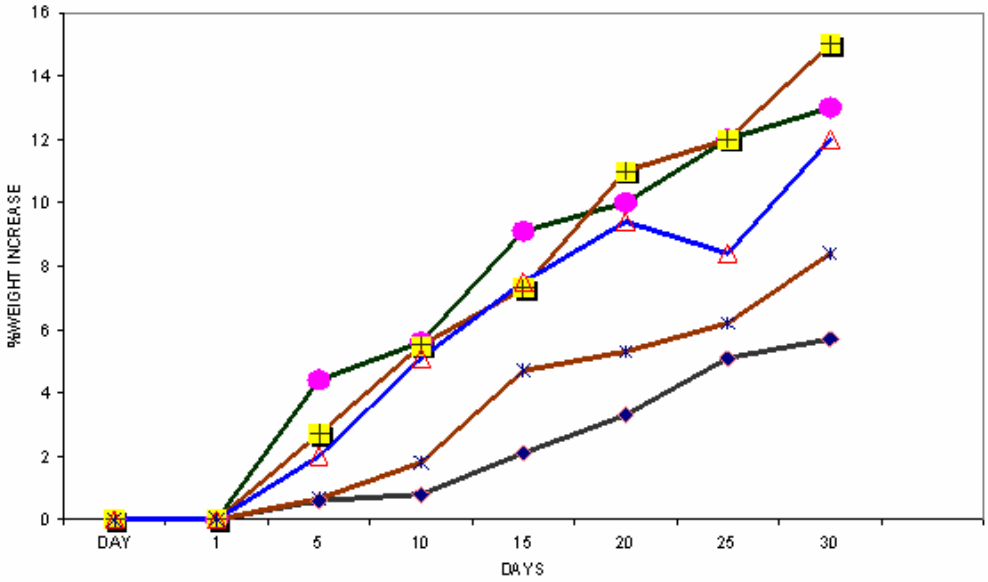

Figure I. Percentage increase in weight of the control and treated animals in the sub chronic study
Key: ▲750mg/kg body weight, ж 500mg/kg body weight, + 250mg/kg body weight, ●100mg/kg body weight and ♦control

The acute toxicity study of the extract indicated no changes in behavior and in the sensory nervous system responses in the animals. Also, no adverse gastrointestinal effects were observed in male and female mice used in the experiment. The median acute toxicity value (LD_{50}) of the extract was determined to be 18.830g /kg body weight. According to Pascoe (1983) and Loomis et al, (1996), the extract can be classified as being non toxic, since the LD_{50}

was found to be more than 15.0g/kg. The gram equivalence of the LD_{50} in an average adult man would translate to 1100g dose of the drug. This is a very high value and makes the preparation relatively safe for use. The viscera of the dead animals did not show any macroscopic changes that could point to the cause of the death. However, since the animals did not convulse before dying, it could be postulated that the extract did not kill the mice by some action on the nervous system (Ogwal-Okeng et al., 2003).

Table III: The effects of the Extract on Kidney, Heart, Liver and Brain in Control and the Treated Groups in the Sub-chronic toxicity Study

Organ	Control	100 mg/kg	250 mg/kg	500 mg/kg	750 mg/kg
Heart (g/100g)	0.33 ± 0.02	0.32±0.05	0.31 ±0.01	0.33±0.01	0.32±0.03
Kidney (g/100g)	0.34 ± 0.04	0.33±0.03	0.33±0.05	0.35±0.04**	0.34±0.01
Liver (g/100g)	3.22 ± 0.02	3.47 ±0.41	3. 23±0.17	3. 61.± 0.21	3.34 ±0.01
Brain (g/100g)	0.77± 0.07	0.76±0.10	0.72±0.08	0.88±0.03**	0.81±0.04

Mean ± SEM, (n=5), *p<0.05; ** p<0.01 vs. control group. Control group received 0.5mL normal saline.

Table IV: Effect of daily administration of the drug for 30 days on biochemical profiles of control and treated rats in the sub-chronic toxicity study

Parameter	control	100 mg/kg	250 mg/kg	500 mg/kg	750 mg/kg
Glucose (mg/dl)	50.60±0.12	87.62±0.62	58.20±0.1**	49.06±0.32*	43.06±0.32*
Cholesterol (mg/dl)	34.60±0.60	45.82±0.03*	40.58±0.42	30.04±1.40**	22.06±0.32*
Triglyceride (mg/dl)	50.60±0.45	30.69±1.50*	18.56±0.02*	12.37±0.02*	16.06±0.32*
HDL (mg/dl)	49.31±0.002	48.5±0.007	308.67±0.07*	180.39±0.49*	350.06±2.50*
LDL (mg/dl)	10.86±2.50	3.75±2.55	very low	very low	very low
Protein (g/dl)	4.06±0.32	4.63 ±0.25	3.68±0.60	6.04±0.20*	6.93±0.32*
Creatinine (mg/dl)	0.86±0.003	0.09±0.001	0.73±0.05	0.95±0.01	1.54±0.32*
AST (IU/L)	19.18±1.20	21.00±2.50**	24.01±0.02*	23.50±2.30*	26.06±0.32*
ALT (IU/L)	52.54±1.80	16.60±2.52*	17.20±2.60*	27.00±3.60*	38.06±0.32*

Mean ± SEM (n =5), *p<0.05; ** p<0.01 vs. control group. Control group received 0.5mL normal saline solution.

Table V. Effect of the drug on haematological parameters of the control and treated animals in the sub-chronic toxicity study

Parameter	control	100 mg/kg	250 mg/kg	500 mg/kg	750 mg/kg
Haemoglobin (mg/dl)	12.20 ±0.3	13.60± 0.40	11.80± 0.40	13.79± 0.40	14.79± 0.40
RBC (10^6 /mm^3)	5.67±0.04	5.07±0.06	6.28±0.05	6.19±0.06	6.52± 0.60
WBC (10^3 /mm^3)	12.30±0.03	6.60±1.65	11.40±0.02	8.08±0.02	9.79± 0.50
PCV %	43.0± 2.20	43.05± 1.52	50.85± 2.50	43.55± 0.50	41.79± 0.10
Calcium (mg/dl)	7.31±0.04	9.05± 0.05	8.58± 0.04	8.24± 0.03	8.41± 0.20
Phosphorus (mg/dl)	11.02± 0.03	10.31± 0.95	11.94± 0.001	11.47± 0.04	11.05± 0.40

*Mean ± SEM (n = 5),*p<0.05; ** p<0.01 vs. control group. Control group received 0.5mL normal saline solution*

The increase in weight was remarkable in the treated animals that received the drug dose of 250mg/kg bwt. This was clearly shown in the mean percentage increase in the weights of the treated animals compared with the control (figure 1). This observed increase in the weights might be attributed to the stimulation of the animals' appetite by the extract which was highest at the dose of 250mg/kg body weight and declined thereafter with the increase in the dose. There were no changes in the colour of the organs of the animals treated with various doses of the drug. Also significant changes in the various organs weights occurred only in the animals that received the highest dose of the

extract. Since no changes in animal behavior and in organs colour were observed at all doses used, the extract or its herbal formulation could be claimed to be non-toxic to the observed organs.

The drug had remarkable decreasing effects on the plasma glucose level especially at the highest dose in the treated rats compared with control. This indicates the presence of hypoglycemic components in the drug. This observation gives credence as to the use of the phytomedicine as a hypoglycemic agent. Increase in the plasma protein level may be a sign of impaired renal function (Kachmar and Grant, 1982), while the elevation in the plasma creatinine concentration indirectly suggested kidney damage, specifically by renal filtration mechanism (Wasan et al., 2001). The increase in the protein and creatinine levels occurred only in the animals that received the highest dose of the extract (750mg/kg body weight). This is a clear indication that the drug at this dose and beyond may cause kidney damage.

The liver releases alanine aminotransferase (ALT) and an elevation in its plasma concentration is an indicator of liver damage. The liver and heart release AST and ALT, with an elevation in plasma concentrations indicating liver and heart damage (Crook, 2006; Wasan et al., 2001). Decrease in ALT and increase in AST levels observed in all the treated groups compared with the control implied that the extract might not have caused some toxic effects on the liver but might have some deleterious effects on the heart tissue (Bürger et al., 2005). The decrease in the plasma total cholesterol (TC) and triglyceride (TG) levels might be attributed to the presence of hypolipidemic agents in the phytomedicine.

The increase in HDL-cholesterol levels and a reduction in LDL-cholesterol levels observed in all the treated animals are an indicator that the drug can reduce the cardiovascular risk factors which contribute to death of diabetic subjects (Barnett and O'Gara, 2003; Ellefson and Caraway, 1982). The reduction of the cardiovascular risk factors gave further support to the traditional use of the herbal formulation of *M. paradisiaca* as a hypoglycaemic agent. Since significant increase in creatinine and protein levels were observed in the animals that received the highest dose of the extract (750mg/kg b.wt.), it implies that the drug at this dose could cause kidney damage (Bürger et al., 2005; Shah Ayub et al., 1997; Klaasen et al., 1995).

Conclusion

The high LD50 value (18.84g/kg) obtained was a clear indication that *M. paradisiaca* herbal preparations could be safe for use. The study showed that the phytomedicine had some hypoglycemic activity and good reducing effects on cardiovascular factors. The study also revealed that the drug at doses investigated did not provoke toxic effects to the animals' liver, but could cause kidney damage which might lead to renal failure on a long term treatment. Kidney functions should therefore be monitored on a long term treatment of diseases with *M. paradisiaca* preparation.

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AUTHORS' CONTRIBUTIONS

The experiment was conceived and designed by Jonathan E Emordi, Steve O Ogbonnia, and Sunday O Olayemi, while the experiments was conducted by Jonathan E Emordi, Steve O Ogbonnia , Sunday O Olayemi, Emmanuel N Anyika, and Chioma Dozie-Nwanna. Paper writing was done by Jonathan E Emordi and Steve O Ogbonnia.