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Assessment of acute impacts of cadmium chloride exposure on blood sugar level, body and organs weight in male albino Wistar rats and the ameliorative effects of ethanol leaf extracts of *Tapinanthus bangwensis* and *Mangifera indica*

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Abstract

Impacts of acute cadmium chloride exposure on blood sugar level, body and organs weights in male albino Wistar rats and the ameliorative effects of ethanol leaf extracts of *Tapinanthus bangwensis and Mangifera indica* were assessed. Twenty –five animals (125- 285 g) were randomly assigned five groups of five rats each. Groups 1-4 were orally administered with cadmium chloride (30%) below its LD_{50} each for three times at 2days interval before treatment with the plant extracts. Groups 1 and 2 were later treated with 300 mg/kg of *T. bangwensis* and *M. indica* respectively. Group3 were treated with 300 mg/kg of both extracts at 50:50 dosage ratio. Groups 4 and 5 were not treated and they served as negative and normal control respectively. Treatment was done via oral route for 14 days and all animals were allowed free access to commercial rat mash and water. Blood sugar and body weight were assessed at 2days interval after exposing animals to the heavy metal followed by treatment. Organs weights were taken after 14days treatment. The results of the blood sugar level revealed significant increase ($P< 0.05$) in group 4 (untreated) when compared to the normal control group and the groups treated with leaf extracts. Significant decrease was recorded in body weight of the untreated rats when compared to the treated and normal groups. There was significant decrease ($P< 0.05$) in the weight of testis and liver in group 4 when compared to groups 1,2,3 and 5, with significant decrease ($P< 0.05$) in the weight of the kidneys in group 1 when compared to groups 3 and 5. These results implied that cadmium chloride toxicity altered blood sugar levels, body and organs weights in the animals, however, the two plant extracts ameliorated these impacts and regulated the parameters to fall within their normal ranges.

Keywords: Cadmium; *Tapinanthus bangwensis; Mangifera indica*; Blood sugar; Body weight; Organ weight

1. Introduction

Nature has presented to humanity the gift of vast therapeutic workshop with wide varieties of medicinal plants. According to WHO more than 80 percent of the global population relies primarily on traditional based medicine to meet up their daily health needs and have encouraged such practice especially where access to conventional treatment is not available or inadequate [1]. However, the inadequate quality control, efficiency and safety validation of medicinal plants has raised concerns over the last decades [2]. Therefore, safety/toxicity evaluation of these plants using animal models is widely encouraged, since the responses by these animals to chemical agents could be translated to human subjects.

Tapinanthus bangwensis is a species of hemiparasitic plant in the tropics of Western sub-saharan Africa. It is woody aerial shrub that is attached to its host plant by hustoria. The presence of antibacterial properties in mistletoe is a great importance in healthcare delivery system, since it could be used as an alternative to orthodox and antibiotics, in the treatment of infections due to the microorganism [3], and will reduce the cost of obtaining healthcare. *Tapinanthus*

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bangwensis have been reported to have phytochemicals properties of flavonoids, lectins, polypeptides, triterpenes, alkaloids, tannins, saponins, steroids, and polyphenolic compounds [4]. Phlobatannins, anthraquinones, besides cardiac and steroidal glycosides have also been reported. The plant has also been reported to treat mental conditions, sterility and health problems associated with urinogenital system, rheumatism and pains [5,6,7]. Mistletoe preparation have been used medicinally for centuries to treat epilepsy, infertility and arthritis. It has been used to treat cancer [8]. It is also reported that *T. bangwensis* leaf extract can treat hypertension, cancerous diabetes or used as a diuretic agent [9,10].

Mangiferia india (mango) is a species of flowering plant in the family Anacardiaceae [11]. Mangiferin, a pharmacologically active hydroxylated xanthone C-glycoside, is extracted from mango at high concentrations from the young leaves (272 g/kg), and from old leaves 94 g/kg) [12]. Other phytochemicals in mango include polyphenols, terperiods, carbohydrates, sterols, carotenoids, vitamins, fatty acids and amino acids, total phenolic compounds, including phenolic acids, Xanthones, benzophenones, tannins, terpeniods and flavonoids [13,14]. *M. indica* extracts have high potential in terms of biological and pharmacological activities viz, antioxidant, antidiabetic, anti- inflammatory, anti- viral, immunomodulatory, anti-obesity, anti-allergic, anti-fungal, anti-parasitic, antidiarrheal, antipyretic and anti-tumor activities [2,13,15].

Heavy metals are ubiquitous and generally in the environment. Biological accumulation in the food chain enable multi-heavy metal contaminant to magnify [16]. The fact is that human beings are normally exposed to multiple heavy metals simultaneously through air, water or food, but not individually. According to [17], exposure to cadmium caused increase in organ weight in albino rats when compare to control. The mechanism of toxicity of cadmium is related to inducement of over production of reactive oxygen species (ROS) and consequently enhanced lipid peroxidation, it causes liver and kidney dysfunction [17]. [18] reported that continuous administration of cadmium on experimental animals caused a significant reduction in body weight and organ weight of the animals. However, this was why investigation was carried out to evaluate the ameliorative impacts of ethanol leaf extract of *T. bangwensis* and *M. indica* on blood sugar, body and organs weights in cadmium exposed male albino Wistar rats.

2. Materials and methods

2.1. Collection and Preparation of Plant Samples

The fresh leaves of *Tapinanthus bangwensis* and *Mangiferia indica* were collected at various locations within Ikot Ekpene and Ibiono Ibom, Akwa Ibom State, Nigeria. The two plants were authenticated by a taxonomist in the Department of Botany and Ecological studies, Faculty of Science, University of Uyo, Akwa Ibom State, Nigeria [19].The two plants leaves were plucked from their stems, washed with distilled water to remove dirt, sliced separately with knife into tiny pieces and dried separately at room temperature for 3 days the dried leaves were later ground separately using clean, dry mortar and pestle and 500g each of the samples were soaked in 150 ml of 70% ethanol for 72 hours at room temperature. The macerated leaves extracts were differently filtered using Whatman No. 1 filter paper by means of funnel. The filtrates were separately concentrated for 3 days, after which slurry form of the extracts obtained and preserved in a refrigerator at 4°C for further use [19].

2.2. Procurement and Preparation of Heavy Metal

The heavy metal (cadmium [Cd]) was obtained in the salt form (cadmium chloride) from the Chemistry unit of the Department of Science Laboratory Technology, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene, Akwa Ibom State, Nigeria. It was stored in an airtight container protecting it from sunlight and moisture [19]. Thirty (30) percent below the LD_{50} of the metal was weighed out, dissolved in 4 ml of distilled water to be administered each on groups 1 to 4 animals for three days at 2days interval before treatment with the leaf extracts.

2.3. Experimental Design, Grouping and Treatment of the Animals

A total of twenty- five (25) healthy adult male albino Wistar rats weighing (125- 285 g) were obtained from the disease free stock of the animal house, Biochemistry Unit, Department of Science Laboratory Technology, Akwa Ibom State Polytechnic, Ikot Osurua. The animals were housed in a cage with sizeable compartments of wooden bottom and wire mesh top, and were randomly assigned five animals per five groups. The rats were maintained under standard conditions of temperature and natural light- dark cycle for 7days acclimatization in the animal house, Akwa Ibom State Polytechnic, Ikot Osurua. The use of animals for the study was carried out in accordance with guidelines set by Institute for Laboratory Animal Research [20]. Groups 1 to 4 animals were exposed to 30 percent below LD_{50} of cadmium Chloride for 3 times at 2days interval based on the average body weights of the groups. Group 1 and 2 animals were later treated with 300 mg/kg *Tapinanthus bangwensis* and *Mangifera indica* leave extracts respectively. Group 3 animals

were treated with 300 mg/kg combined extracts of the two leaves at 50:50 dosage ratio. Group 4 and 5 were not treated with either of the leave extracts and served as negative and normal control respectively. Treatment was done daily via oral route for a period of 2weeks.

2.4. Methods

2.4.1. Determination of Blood Sugar Level

Blood sugar levels in the animals was determined using glucometer. A sterile lancet was used to prick the tip of the tail of each animal to obtain blood after overnight fighting. The blood was introduced into the instrument (glucometer) by means of a fine test strip, after which the glucose level was read in mg/dl. This test was conducted at 2days interval for a period of 2weeks.

2.4.2. Determination of body and organ weights

The body weight of the rats was taken with a weighing balance. Each animal in each group was weighed 3times a week at 2days interval for the period of 2weeks in grams. The weight of organs (testis, kidney and liver) were obtained after 14days, after which the organs were harvested and weighed using a sensitive weighing balance.

2.4.3. Statistical Analysis

Data obtained from the test were subjected to one one-way analysis of variances (ANOVA). Statistical significant differences were obtained at ($p<0.05$) by Bonferoni multiple range test. The results expressed as mean±standard error of mean (SEM) estimated using statistical package of social science (SPSS) version 26.

3. Results

Table 1 Mean blood glucose level of male albino Wistar rats exposed to cadmium and treated with ethanol leaf extracts of *Tapinanthus bangwensis* and *Mangifera indica* for 14days

Groups		**1**	**2**	**3**	**4**	**5**
Blood Glucose (mg/dl)	Initial Blood Glucose	119.00±20.03*	75.20±4.41	65.80±10.40*	89.60±7.75	84.8±11.87
	Day2	73.00±10.19	74.80±4.78	75.40±8.79	78.00±13.31	79.00±6.46
	Day4	95.20±12.63	79.00±3.21	91.80±10.28	132.80±11.06*	80.60±4.35
	Day6	97.80±13.99	97.00±8.11	100.20±9.87	141.00±2.24*	75.80±4.85
	Day8	69.80±1.83	62.00±3.43	71.00±2.09	85.60±10.76*	65.60±4.49
	Day10	70.80±2.95	68.20±1.49	72.00±2.44	88.20±10.80*	68.00±3.86
	Day12	77.20±3.48	65.20±5.60	77.8-±4.94	96.00±6.57*	72.20±2.38
	Day14	91.00±16.93*	65.60±5.20*	71.60±2.50	90.00±8.04*	80.40±3.64

Result presented as mean ± standard Error of Mean (SEM) of triplicate; Values with asterisk indicates statistical significance at $p<0.05$

Table 2 Mean body weight of male albino wistar rats exposed to cadmium and treated with ethanol leaf extracts of *Tapinanthus bangwensis* and *Mangifera indica* for 14days

Groups	Body weight(g)							
	Initial Body weight	**Day2**	**Day4**	**Day6**	**Day8**	**Day10**	**Day12**	**Day14**
1	163.80±16.80	155.80±14.57	163.20±11.50	159.4±11.63	164.00±11.40	159.40±12.01*	201.20±18.00	200.40±19.99
2	195.40±20.28	176.00±18.47	193.00±18.01	185.40±16.71	185.40±16.71	186.00±14.03	196.80±17.19	190.60±18.20
3	207.20±16.58	203.00±15.85	200.80±13.81*	163.60±13.21	176.60±13.33	178.80±13.99	206.40±13.74	203.60±17.19*
4	151.40±21.31	153.60±12.11*	166.40±12.69*	156.00±14.91	173.80±12.11	177.60±11.95	158.40±12.30*	189.80±13.32*
5	177.40±12.92	199.00±14.23	188.60±14.39	180.00±12.20	188.20±14.50	185.80±16.28	200.20±15.16	199.20±15.27

Result presented as mean ± standard Error of Mean (SEM) of triplicate; Values with asterisk indicates statistical significance at p<0.05

Table 3 Mean organs weight of male albino Wistar rats exposed to cadmium and treated with ethanol leaf extracts of *Tapinanthus bangwensis* and *Mangifera indica* for 14days

Groups	Organs weight (g)		
	Testis	**Kidney**	**Liver**
1	2.24±0.05*	1.23±0.07*	7.44±0.40
2	2.49±0.02	1.41±0.04	7.89±0.32
3	2.64±0.11*	1.49±0.01	8.78±0.18
4	2.53±0.07	1.33±0.10*	6.31±0.44*
5	2.54±0.05	1.49±0.07	7.93±0.23

Result presented as mean ± standard Error of Mean (SEM) of triplicate; Values with asterisk indicates statistical significance at p<0.05

4. Discussion

Medicinal plants are considered as rich resource of ingredient which can be used in drugs development, either pharmacopoeial, non-pharmacopeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world. Medical plants have been resources for healing in local communities around the world for thousands of years [8]. According to researchers, plants are indispensable sources of both preventive and curative medicines. Hundreds of plants species are recognized for their therapeutic values and are used to treat various ailments [10]. The use of these plants may be due to their less toxic effects, relative availability and less expensive compared to other chemotherapeutic formulations. Several reports have been made on the pharmacological potential of *Tapinanthus bangwensis* and *Mangifera indcia* to include, antioxidant, antimicrobial, antidiabetic, anti-inflammatory, hepatoprotective etc. [11,21,22,7]. However, it was highly crucial for the functional architecture of blood glucose level, body and organs weights, particularly the kidney and liver of male albino wistar rats to be evaluated as the animals were exposed to cadmium, then treated with ethanol leaf extracts of *Tapinanthus bangwensis* and *Manifera indica.*

Meanwhile, the results obtained in this work revealed that blood glucose level in group1 before exposure to heavy metal (cadmium) showed statistical significant increase when compared to groups 2 and 3, but non-significantly different when compared to groups 4 and 5. On days after exposure, no significant difference was observed between the groups

and within the groups. On day 4, group 4 recorded significant increase in the mean serum blood glucose level when compared to groups 1,2,3 and 5 respectively but there was no significant difference among other groups. On day 4,6,8,10 and 12, group 4 recorded significant increase in the mean serum blood glucose level when compared to all other groups, but on day 14, no significant difference was found between groups and within groups of animals. However, the values of blood glucose levels in the animals were within the normal blood glucose level (72-108 mg/dl) for human except in group 4 that was exposed to cadmium without treatment with either of the plant extracts. The observed increase in the glucose level was dependent on the duration of exposure of the animals to the heavy metal (cadmium). This observation agrees with the report by [23], that significant decrease in serum glucose level from 305.40± 9.68 mg/dl to 210.10± 2.03 mg/dl was obtained when aqueous extract of *T*. *bangwensis* leaf was administered on experimental animals exposed to lead acetate. Another report by [24] asserted that oral administration of *M*. *india* seed and leaf extracts to $CdCl_2$ exposed rats significantly reduced the blood glucose levels. According to the report, the glucose level reduced from 255.50± 2.36 mg/dl to 130.60± 6.93 mg/dl when 400 mg/kg of the leaf extract was administered and from 426.50±13.08 mg/dl to 100.50± 7.8 mg/dl when 400 mg/kg of seed extract was administered.

The glucose concentration observed in this study described an abnormally low level of blood glucose (Hypoglycemia). Hypoglycemia is not a disease in itself, but it is a sign of a health problem which may be because of the glucose lowering activity of the extracts and the heavy metal (cadmium), which may have been inhibitive to activity of glucose formation. The plants leave extracts might have lowered the glucose level by stimulating glucose metabolizing enzymes, increasing gluconeogenesis and inhibiting glycogenolysis. The treatment of the animals with the plant extracts after exposing them to the heavy metal might have achieved the glucose lowering activity by decreasing rate of carbohydrate absorption into the hepatic portal circulation, increase glucose transport and uptake and increase insulin secretion.

Furthermore, the results of the body weight revealed that the initial body weight of the animals before exposure to cadmium was significantly increased in group 3 when compared to all other groups. On day 1 after exposure, significant decrease was observed in group4 when compared to group 3 and when compared to group 5. On day 4, there was no significant difference in the body weight in group 3 when compared to groups1 and 4. On day6, a non-significant difference was shown in groups 1 and 4 when compared to group2. Day 8, 10 and 14 recorded no significant difference in the body weights of the animals between the groups and within the groups. However, on day 12, significant decrease in the body weight was found in group 4 when compared to group3. No significant difference was found in the body weight of the animals on day 14. This results supports the report by [25] that after 60 days of daily oral administration of an aqueous and ethanol extracts of *T. bangwensis* seeds on experimental animals, there was a general increase in body weight in all groups. The body weight changes observed between the groups were not statistically significant when compared to the control. [26] reported that the body weight of experimental animals did not show any significant changes after treatment with an aqueous leaf extract of *M. india* to albino rats when compared to their control group. [27] reported significant decrease in body weights of rats given 0.1% lead acetate at the third month of treatment, when compared to the control group and it was normalized later. The weight decrease may be attributed to the loss in muscle mass and adipose tissue resulting from excessive breakdown of tissue fats and protein. Changes in body weight is related to changes in organ weight depending on organ involved in the metabolism of a particular substance [26]. On the basis of organs weights of the animals sacrificed after the 14^{th} day treatment, group1 animals recorded significant decrease in the mean testis weight when compared to groups 2,3,4 and 5. Kidneys weights showed significant decrease in group1 when compared to groups 3 and 5 respectively. [26] reported no significant difference in organ weight of albino Wister rats after administration of aqueous leaf extract of *M*. *indica* for 28 days when compared to the control group. The present study has shown that the heavy metal (cadmium) has an altering effects on the blood sugar level, body and organ weights of the albino Wistar rats. However, the leaves extracts administered rendered some ameliorative effects on the damaged organs caused by the heavy meal. This significant changes observed is suggested to be due to the active metabolism of the endogenous molecules and the phytoconstituents of the two plants extracts which exhibited marked regulatory properties on blood sugar levels, body and organs weights in the animals.

5. Conclusion

Acute effects of cadmium exposure on blood glucose levels, body and organs weight in the study of animals were observed. However, the ethanol leaves extracts of *Tapinanthus bangwensis* and *Mangifera indica* might have been implicated in the regulation of toxic effect of the heavy metal on those parameters thereby making them to fall within their respectively normal ranges.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

No ethical conflicts exist with the use of animal in this work.

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