# Evaluation of Acute Effects of Heavy Metal Exposure on Serum Total Protein, Albumin and Globulin in Male Albino Wistar Rats Treated with Ethanol leaf Extracts of *Tapinanthusbangwensis*and *Mangifera indica*


## ABSTRACT

Acute impacts of heavy metal exposure on serum total protein (TP), albumin (AL) and globulin (GL) in male albino wistar rats treated with ethanol leaf extracts of *Tapinanthusbangwensis*and *Mangifera indica* were evaluated. Twenty-five (25) animals (125-250g) 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 days at 2 days interval before treatment with the plant extracts. Groups 1 and 2 were later treated with 300mg/kg of *T. bangwensis* and *M. indica* respectively. Group 3 was treated with 300mg/kg combined extracts of the two plants at 50:50 dosage ratio while group 4 and 5 were treated with the plant extracts and served as positive and normal control respectively. Animals in each group were allowed free access to commercial rat mash and water throughput two weeks of treatment which was done daily via oral route. After the fourteen (14) days experimentation, the results revealed a significant increase ($P<0.05$) in serum total protein in group 2 and 3 when compared to group 1. No significant difference($P>0.05$) was observed in group 1 compared to the positive and normal controls. Group 2 showed significant increase ($P<0.05$) when compared to groups 3 and 5. Group 3 showed significant increase ($P>0.05$)when compared to groups 4 and 5, but with no significant difference ($P>0.05$) in group 4 compared to group 5. Serum albumin level showed significant increase ($P<0.05$) in group 1 when compared to group 2 and 3, but with no significant difference ($P<0.05$) compared to groups 4 and 5.Significant decrease in group 2 ($P<0.05$) when compared to group 4. Group 4 compared to group 5. Significant increase in serum albumin was revealed in group 4 when compared to group 5. Serum globulin recorded significant decrease ($P>0.05$) in group 1 compared to group 2 and 3, but with no significant difference ($P<0.05$) compared to group 4and 5. Group 2 showed significant increase ($P<0.05$) when compared to groups 4 and 5. Group 3 recorded significant increase ($P<0.05$) in serum globulin when compared to Group 4, but with no significant difference ($P>0.05$) compared to group 5. The implications of these resultsare discussed.


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**Keywords:** Evaluation, Acute effect, Heavy metal, total protein,Albumin, Globulin, Albino Wistar rats.



## INTRODUCTION

Medicinal plants are integral part of human life to combat the sufferings from the dawn of civilization (Chaudhary *et al.*,2010). It is estimated that more than 80,000 of total plants species have been identified and used as medicinal plants around the world. The indigenous medicinal plants and plant-derived drugs are the potential source of alternative

medicine and are extensively used to treat various health ailments (Karishankar *et al.*,2011). Use of medicinal plants is a core component of primary health care level due to availability, acceptability, compatibility and affordability. Some of these medicinal plants whose efficacy are reported are *Tapinanthus bangwensis* and *Mangifera indica*.

*Tapinanthus bangwensis* (mistletoe) belongs to the family, Visceceae. It is a well-known ever green parasitic plant which grows on deciduous trees in ball-like bush (Evans, 2005). It is an excellent medicinal plant which have been used against a variety of diseases such as disorders in female reproductive system, cancer, arthritis, rheumatism,epithermal tumors, hypertension, asthma, nervousness and epilepsy (Evans 2005; Oswala *et al.*, 2005; Edet *et al.*, 2011). Mistletoe do grow on either edible or non-edible tress. But it is reported that those that grow on edible trees have disease curing specificity, for example mistletoe grown on guava, kolanuts and citrus are specific for curing diseases like cancer, hypertension, nervousness and insomnia, while those growing on cocoa is best used for curing diabetes.

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*Mangifera indica*(mango) is juicy stone fruit which belongs to a family Anacardiaceae in the order sapindales, and is grown in many parts of the world, particularly in tropical countries. *M. indica* is one of the flowering plants which is one of the most popular tropical fruits rich in a polyophenolic antioxidant and glycosylxanthone that makes it has strong antioxidant, antilipid peroxidation, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic properties and other medicinal effects including antibacterial, antifungal, ~~anthelminitic~~anthelmintic, antimicrobial and gastroprotective activities (Tharanthan *et al.*, 2006). However, the rich medicinal properties reported on these two plants prompted the evaluation of acute effects of cadmium exposure on serum total protein, albumin and globulin in male albino ~~wistar~~ Wistar rats treated with ethanol leaf extracts of *Tapinanthus bangwensis and Mangiferaindica*.



## MATERIALS AND METHODS

### Collection and Preparation of Plant Samples

The fresh leaves of *Tapinanthus bangwensis and Mangifera indica* were collected at difference locations within Ikot Ekpene and Ibiono Ibom Local Government Areas, both in 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.

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 400g each of the samples were soaked in 100ml 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 days, after which slurry form of the extracts obtained and preserved in a refrigerator at $4^0$C for further use.

### 2.2 Procurement and Preparation of Heavy Metal

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

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### 2.3 Experimental Design, grouping and Treatment of the Animals

A total of twenty-five (25) healthy adult male albino ~~wistar~~ Wistar rats weighing (125-258g) were obtained from the diseasefree stock of the animal house, Biochemistry Unit, Department of Science Technology, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene, Akwa Ibom State, Nigeria. The animals were housed in a cage with five sizeable compartments of wooden bottom and were mesh top, randomly assigned five animals per five groups. The rats were maintained under standard conditions of temperature and natural light-dark cycle for 7 days acclimatization in the animal house, Akwa Ibom State Polytechnic, Ikot Osurua. Groups 1 to 4 animals were treated with 30 percent below $LD_{50}$ of cadmium chloride for 3 days at 2 days interval based on the average body weights of the groups. Group 1 and 2 animals were later treated with 300mg/kg *Tapinanthus bangwensis and Mangifera indica* leave extracts respectively Group 3 animals were treated with 300mg/kg combined extracts of the two plant leaves of 50:50 dosage ratio.Groups 4 and 5

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were not treated with either of the leave extracts and served as positive and normal control respectively. Treatments were done daily via oral route for a period of 2 weeks.

### 2.4 Collection of Blood Sample and Preservation of Serum

At the end of 7 days experimental period, the animals were fasted for 12 hours and were anaesthetized under chloroform vapour and were sacrificed by dissecting medioventrically and blood was collected via cardiac puncture by means of syringe and needle into a sterile EDTA sample bottles and then centrifuged at 3,000rpm for 15 minutes to separate serum from the plasma. The serum was used to determine the levels of Total protein, Albumin and Globulin in the animals.

### 2.5 Methods

#### 2.5.1 Determination of Total Protein

Total protein level was determined based on the method described by Tietz (1995). Standard commercial kid supplied by Randox Laboratories Ltd, UK was used. Twenty microliters (20µl) each of the distilled water, standard and serum sample were pipetted into respective test tubes labeled reagent blank, standard and sample. Then, 1000µlof biuret reagent ($R_1$) was pipetted into each of the tubes, mixed and incubated for 30minutes at $25^0$C. The absorbance of the sample and standard were read against the blank at 546nm with a spectrophotometer. The total protein concentration was calculated using the formula;

$$\text{Total protein (g/l)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard}$$

#### 2.5.2 Determination of Albumin

The concentration of albumin was determined based on the method by Grant *et al.*(1994). Ten microliters (10µl) each of distilled water, standard and serum sample were pipetted into three separate test tubes labeled blank, standard and sample respectively.Thereafter, 3000µl of Bromo Cresol Green (BCG) reagent ($R_1$) was measured into each of the test tubes, mixed and incubated at room temperature for 5 minutes after which the absorbance of the sample and standard were measured against the absorbance of the blank at 578 nm with a spectrophotometer. Albumin concentration was calculated using the formulated;

$$\text{Albumin concentration (g/L)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard}$$

### 2.5.3 Determination of Globulin

Globulin was determined as the difference between total protein and albumin.

Globulin concentration (g/L) = Total protein– Albumin

## 2.6 Statistical Analysis

Data obtain from the analysis of subjected to one-way analysis of variance (ANOVA). Statistical significant differences were obtained at ($p>0.05$) by Bonferroni's multiple range test. The results expressed as mean ± standard error of mean (SEM) estimated using statistical package for Social Science (SPSS) version 23.

## 2.7 Results

**Table 1:Mean Acute Effects of Heavy Metal Exposure on Serum Total Protein, Albumin and Globulin in Male Albino Wistar Rats treated with Ethanol leave Extracts of *Tapinanthusbangwensis*and *Mangifera indica***

| Group | Total Protein (g/L) | Albumin (g/L) | Globulin (g/L) |
|---|---|---|---|
| 1 | 75.55± 0.69 | 38.33± 0.64 | 36.85± 0.85 |
| 2 | 79.43± 2.72 | 36.98± 0.73 | 42.52± 2.31 |
| 3 | 80.91± 2.79 | 36.80± 0.84 | 41.92± 2.81 |
| 4 | 76.54± 2.16 | 36.08± 1.17 | 37.44± 2.88 |
| 5 | 77.31± 1.20 | 37.43± 1.14 | 39.51± 1.88 |

Result presented as mean ± SEM, N = 5

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## 2.8 Discussion

The use of medicinal plants in most developing climbs is a development that has attracted more concerns among healthcare workers and researchers. According to researches, plants are indispensable sources of both preventive and curative medicines (Lid *et al.*, 2020).Several reports have been made on the pharmacological potentials of *Tapinanthusbangwensis*and *mangifera indica* to include, antioxidant, antimicrobial, antidiabetic, anti-inflammatory, hepatoprotective etc., properties (Bharti, 2013). The present study sought to evaluate the acute effects of cadmium chloride exposure on serum total protein, albumin and globulin in male albino wistar rats treated with leave extracts of

*T. bangwensis* and *M. indica*. The results revealed that group 2 animals and group 3 showed statistical significant increase in serum total protein levels when compared to group 1. No significant difference was observed in serum total protein level in group 1 when compared to group 4 (positive control) and when compared to the normal control group(Group 5). Group 2 animals recorded significant increase when compared to group 4, but with no significant difference when compared to groups 3 and 5. Groups showed significant increase when compared to group 4 and 5. Meanwhile, serum total protein levels in treatment and control groups fell within the normal range (60-80g/L) for human subject (Geyer *et al.,* 2016). Though group 3 recorded a raised serum total protein (80.90g/L) which was slightly above the upper normal limits, this might have been caused by synergistic effects of the combined treatment. The result is in accordance with Malani*et al.* (2014) who reported an increase serum total protein and albumin levels in $CCl_4$ induced hepato-toxic rats treated with aqueous stem-bark extract of *M. indica*. The least serum total protein (75.55g/L) in this study was found in group 1 treated with *T.bangwensis*leaf extract. This finding suggests that *T. bangwensis* leaf extract possesses strong hepatoprotective potential against cadmium chloride induced hepato-toxicity in the rats. The result is in agreement with report by Nwangwu *et al.* (2010) who recorded significant reduction in serum total protein in normal rats treated with leaf extract of*T. bangwensis.* Again, decrease in serum total protein in the positive control group maybe due to heavy metal induced toxicity in the rats. Increase in serum total protein above normal range could be caused by chloric inflammation or infection such as HIV or hepatitis B or C, multiple myeloma, Waldenstron's disease (Smith*et al.*,2013), whereas decrease maybe caused by malnutrition, malabsorption, disorders such as Celiac disease or inflammatory bowel disease (IBD) and liver/kidney diseases (Geyer *et al.,* 2016). Symptoms of total protein deficiency include, weakness, cracked, pitted nails e.t.c., whereas, elevation maybe caused by dehydration, unexplained exhaustion, nausea, irritability, diarrhea, headache (Geyer *et al.,* 2016).

Furthermore, serum albumin level in this work showed significant increase in group 1 when compared with group 2 and 3, but with no significant difference when compared to groups 4 and 5. Significant decrease was shown in group 2 compared to group 3 and 5. Group 4 showed significant increase when compared to group 3. Again, significant increase was recorded in serum albumin level in group 4 compared to group 5. Moreso, serum albumin levels in treatment and control groups fell within the normal limit(30-50g/L) for human. But, the positive control group (group 4) recorded the lowest (36.08g/L).

This agrees with the report by Kumar and Sharma (2021) and Onyinloye*et al.*(2016) who observed reduction in serum albumin due to oxidative stress induced by mercury in normal male rats. The highest serum albumin level in group 1 animals might be as a result of strong antioxidant and hepatoprotective properties of *T. bangwensis*reported by Saraswat *et al.,* (1993). The authors reported that prolong treatment of normal albino rats with *T. bangwensis* leaf extract caused its bioaccumulation and significant increase in serum albumin level leading to hyperalbuminemia, thereby resulting in lever/kidney dysfunction. Meanwhile, increase of serum albumin level above normal could be caused by dehydration which may lead to very high osmotic pressure of the plasma (Harper and Dugauzyk, 2010). Symptoms include, jaundice, severe tiredness, weight loss, fever etc.,(Vasdevan*et al.,* 2011). Decrease level below normal maybe caused by liver disease, heart failure, malnutrition etc., symptoms include, weak muscle, fatigue, kidney disease etc., (Harper andDugauzyk, 2010).

Moreso, serum globulin levels in the work showed significant decrease in group 1 when compared to group 2 and 3, but with no significant difference when compared to groups 4 and 5. Group 2 recorded significant increase when compared to groups 4 and 5, but with no significant difference compared to group 3. But, group 3 showed significant increase when compared to group 4, with no significant difference when compared to group 5. Observably, serum globulin levels in treatment and control groups were within the normal limit (20-35g/L) for human. Group1 animals recorded the lowest level of 36.85g/L, while the highest, 42.52g/L was for group 2. This suggests that the extract (*M. indica*) may be activated and released protein such as antibodies. Decreased globulin recorded in group 1 suggests hepatoprotective activity against toxic effect that might have been caused by the heavy metal resulting in the reduction. This result agrees with Nwangwu *et al.* (2010) who reported significant reduction in serum albumin and globulin on administration of *T.bangwensis* leaf on normal albino rats. High levels of serum globulin could be caused by cirrhosis of the liver multiple myeloma, collagen disease etc., with fatigue, weakness, dizziness, muscle numbness etc., (Garcia *et al.,* 2015) as symptoms. Decrease serum globulin below normal could be caused by liver or kidney disease etc., and symptoms may include, swelling of feet, ankle, leg and/or abdomen (Sanches-Monge *et al.,* 2004).

## 2.9 Conclusion

There was no adverse acute effects of cadmium exposure on serum total protein, albumin and globulin in the study animals. However, the ethanol leaves extracts of *T.*

*bangwensis* and *M.indica* might have been implicated in the regulation of toxic effects of cadmium on the serum proteins.

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