

Evaluation of Acute Effects of Cadmium Toxicity on Serum Total Protein, Albumin and Globulin in Male Albino Wistar Rats Treated with Ethanol leaf Extracts of *Tapinanthus bangwensis* 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 *Tapinanthus bangwensis* and *Mangifera indica* were evaluated. Twenty-five (25) animals (125-250 g) were randomly assigned five groups of five rats each. Groups 1-4 were orally administered with cadmium chloride (30 %) below its LD₅₀, (267 mg/kg) each for three times at 2 days 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. Group 3 was treated with 300 mg/kg combined extracts of the two plants at 50:50 dosage ratio while group 4 and 5 were not 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 throughout 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 4 and 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 results are that human should not be exposed to heavy metals to avoid toxicity, also if exposed the extracts used could be administered to ameliorate

Keywords: Cadmium, total protein, Albumin, Globulin, Albino Wistar rats, *Tapinanthus bangwensis* and *Mangifera indica*.

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, rheumatoid arthritis, 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, kola nuts and citrus are specific for curing diseases like cancer, hypertension, nervousness and insomnia, while those growing on cocoa is best used for curing diabetes.

“*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 polyphenolic antioxidant and glycosylxanthone that makes it has strong antioxidant, antilipid peroxidation, immunomodulation, cardiogenic, hypotensive, wound healing, antidegenerative and antidiabetic properties and other medicinal effects including antibacterial, antifungal, anthelmintic, antimicrobial and gastroprotective activities” (Tharanthan *et al.*, 2006). Acute effects of Cadmium on human health have been reported (Akpakpan *et al.*, 2023; Zhang *et al.*, 2023; Peana *et al.*, 2023).

“However, the rich phytochemical constituents and 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 rats treated with ethanol leaf extracts of *Tapinanthus bangwensis* and *Mangifera indica*” (Sakyiamah *et al.*, 2023; Kumar *et al.*, 2021; Atewolara-Odule *et al.*, 2018).

Therefore, the aim of this work is to evaluate acute effect of cadmium toxicity on total protein, albumin, and globulin in male albino Wister rat treated with *Tapinanthus bangwensis* and *Mangifera indica*.

MATERIALS AND METHODS

Collection and Preparation of Plant Samples

The fresh leaves of *Tapinanthus bangwensis* and *Mangifera indica* were collected at different 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. (Essien et. al. 2022)

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 100 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 days, after which slurry form of the extracts obtained and preserved in a refrigerator at 4⁰C for further use. (Essien et. al. 2022)

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 LD₅₀ 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.

2.3 Experimental Design, grouping and Treatment of the Animals

A total of twenty-five (25) healthy adult male albino Wistar rats weighing (125-258g) were obtained from the disease free 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 exposed to 30 percent below LD₅₀ of cadmium chloride (267 mg/kg) for 3 times at 2 days 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* leaf extracts respectively, Group 3 animals were treated with 300 mg/kg combined extracts of the two plant leaves of 50:50 dosage ratio. Groups 4 and 5 were not treated with either of the leaf 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 14 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 kit 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, 1000µl of biuret reagent (R₁) was pipetted into each of the tubes, mixed and incubated for 30minutes at 25⁰C. 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 (R₁) 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;

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

2.5.3 Determination of Globulin

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

$$\text{Globulin concentration (g/L)} = \text{Total protein} - \text{Albumin}$$

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 \pm 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 *Tapinanthus bangwensis* and *Mangifera indica*

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

Result presented as mean \pm SEM, N = 5

2.8 Discussion

The use of medicinal plants in most developing countries 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 *Tapinanthus bangwensis* 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 leaf 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 CCl₄ 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 chronic inflammation or infection such as HIV or hepatitis B or C, multiple myeloma, Waldenström’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 liver/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 and Dugauzyk, 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 (20-35 g/L) for human. Group1 animals recorded the lowest level of 36.85 g/L, while the highest, 42.52 g/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 were no adverse acute effects of cadmium exposure on serum total protein, albumin and globulin in the study animals. However, the ethanol leaves extract of *T. bangwensis* and *M. indica* might have been implicated in the regulation of toxic effects of cadmium on the serum proteins.

Ethical Approval:

Animal Ethic committee approval has been collected and preserved by the author(s)

REFERENCES

- Akah, P. A. and Okarfor, C. L. (1992). Blood sugar lowering effects *T. bangwensis* experiment model animals. *Phytotherapy Research*, 6:171-175.
- Akpakpan A.E., Inam, E.J. and Itoro E. U., (2023), Preparation, Characterization and Application of Soda Lignin and its Ester Derivatives as Adsorbents in the Adsorption of Pb²⁺ and Cd²⁺ from wastewater, *J. Mater. Environ. Sci.*, 14(1): 82-96
- Atewolara-Odule, O. C.; Aiyelaagbe, O. O. and Ashidi, J. S. (2018) Biological and Phytochemical Investigation of Extracts of *Tapinanthus bangwensis* (ENGL. AND K. KRAUSE) Danser [Loranthaceae] Grown in Nigeria . *African Journal of Science and Nature*, 6,73- 80
- Bharti, R. P. (2013). Studies on antimicrobial activity and phytochemical profile in *M. indica* leaf extract. *Journal of Environmental Science and Toxicological Food Technology*, 7:74-78.
- Chandhary, H., Goyal, S. and Poonia, P. (2010). *Lawsonia inermis* Linneaus: A Phytopharmacological Review. *International Journal of Pharmaceutical Science and Drug Research*, 2(2):91-98.

- Edet, E. C., Akpanbiatu, M. I., Udo, F. E., Eno, A. E and Itam, E. T. (2011). *G. latifolium* crude leaf extract reverse alterations in haematological indices of weight loss in diabetes rats. *Journal of Pharmaceutical and Toxicology*, 6:174-181.
- Evans, J. (2005). Mistletoe: God for more than free kisses. *Journal of American Botanical Council*, 68:50-59.
- Garcia, R., Noivest, L., Sen, S., Mookeryee, R. and Jalan, R. (2015). Albumin infusion improves renal blood flow. Auto-regulation in patient with acute decompensation of cirrhosis and acute kidney injury. *Liver International*, 35(2):335-343.
- Greyer, P. K., Kulak, N. G., Hoidt, L.M., Teupser, D. and Mann, M. (2016). Plasma proteome profiling to access human health and disease. *Cell System*, 2(3):185-195.
- Grant, G. H., Silverman, L. M. and Christenson, R. H. (1987). "Amino Acids and Proteins". *Fundamentals of Clinical Chemistry* (3rd ed.) Philadelphia: W. B. Saunder Company. P. 379.
- Harper, M. E. and Dugauzyk, A. F. (2010) Linkage of evolutionary related serum albumin and alphafero-protein genes within 11-22 of human chromosome 4. *American Journal of Human Genes*, 38(4):565-572.
- Kauishankar, G., Lankshmideus, N., Marthy, S. M., Prakash, H. and Niranjana, S. (2011). Diabetes and medicinal plant. *Journal of pharmaceutical and Biomedical Sciences*, 2(3):65-80
- Kumar, A. and Sharma, B. (2021). Protective effect of eugenol on hepatic inflammation and oxidative stress induced by cadmium in male rats. *Biomedicine and Pharmatherapy*, 139:1115-11160.
- Kumar M, Saurabh V, Tomar M, Hasan M, Changan S, Sasi M, Maheshwari C, Prajapati U, Singh S, Prajapat RK, Dhumal S, Punia S, Amarowicz R, Mekhemar M. (2021). Mango (*Mangifera indica* L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. *Antioxidants* (Basel). 10(2):299.

- Lid, I. I., Kumar, S., Shukla, S., Kumar, V. and Sharma, R. (2020). Putative antidiabetic herbal food ingredient. Natural functional properties, bioactivity and effect on metabolic pathways. *Trends in Food Science and Technology*, 97:317-340
- Malani, I., Musa, M. S., Athassan, A. M., Dallatu M. K. and Abdullahi, K. (2014). Hepatoprotective activity of stem-bark extract of *M. indica* on carbon Tetra chloride induced hepatic injury in Wistar Rats. *International Journal of Pharmacological Science Research*, 5(4):1240-1245.
- Nwangwu, S. C., Iko, S., Olley, J. M., Oke, T. and Uhanmwangho, P. (2010). Effects of ethanol and aqueous leaf extracts of *Landolphra awariensis* on the serum lipid profile of rats. *African Journal of Biochemical Research*, 3:136-139.
- Onyinloye, B. E., Basim, O.A., Ojo, O. A., Musa, M. H. and Ojo, A. A. (2016). Ameliorative potential of *Afromonium melegueta* extract in cadmium induced hepatic damage and oxidative stress in male Wistar rats. *Journal of Applied Pharmaceutical Science*, 10(7):94-99.
- Osuala, F. O., Nnolic, M. C. and Anyadoh, S. O. (2005). Antibacterial activities of two local medicinal plants *G. latifolium* and *O. gratissimum*. *International Journal of Natural and Applied Science*, 1(1): 36-389.
- Peana, M., Pelucelli, A., Chasapis, C.T., Perlepes, S.P., Bekiari, V., Medici, S., Zoroddu, M.A. (2023). Biological Effects of Human Exposure to Environmental Cadmium. *Biomolecules*, 13 (36): 1-19
- Sakyiamah, M.M., Gordon, P.K., Bolah, P. Baffour, k., Ehun , E., Quasie , O., Kumadoh, Archer, M., D., Mintah, S.O. and Appiah,A,A. (2023). Assessment of the phytochemical composition and antimicrobial properties of *Tapinanthus bangwensis* leaves hosted by the branches of *Persea americana*. *BMC Complement Med Ther* 23, 34
- Sanchez-Monge, R., Lopez-Toreejoin, H., Paschal, C., Varela, J., Martin-Esteban, M. and Salado, G. (2004). Medicinal plants of India. *Indian Journal of Medicinal Research*, 120:9-14.

- Saraswat, B., Visen, P. K., Patnaik, L. and Dhawan, N. B. (1993). Anticholestatic effect of *Picrorhiza kurooa* against carbon tetrachloride-induced cholestasis. *Indian Journal of Experimental Biology*, 31:316-320.
- Smith, S. Graham, S. Walter, O., Gail, L. Walter, V., Robin, M., Haschek, P., Wanda, M. and Matthew, A. (2013). Similar Pathology in non-clinical toxicology testing. Haschet and Rouseaves Handbook of Toxicology pathology (3rd. ed.). Boston Academic Press. Pp. 568-594.
- Tietz, N. W. (1995). Clinical guide to Laboratory test (3rd. ed.). W. B. Saunders, Philadelphia. Pp.27-32.s
- Tharanthan, R. N., Tashoda, H. M. and Prabha, T. N. (2006). Mango (*M. indica*) the king of fruits: A review. *Food Reviews International*, 22:95-101.
- Vasudevan, D. M., Strakumari, S. T., Kannan, L. P. and Vaidynathan, U. U. (2011). Text book of Biochemistry for medical students (6th.ed). Malaysia JAPEK. Pp. 330-332.
- Zhang, R, Chen, Z-H, Cui, W-T, Qiu, S-Y, Qian, Z-H, He X-Ge. (2023). Cadmium stress interacts with nutrient availability and light condition to affect the growth of *Hydrocotyle vulgaris*. PLoS ONE 18(1): 1-12
- Essien NE, Bobson PG, Ukpong EG, Essien U, Okon OE, Nyah N. Evaluation of the Effects of Treatment of Normal Albino Wistar Rats with Ethanol leaves extract of *Mangifera indica* and *Gongronema latifolium* on the Lipid Profile. *International Journal of Research in Engineering and Science (IJRES)*. Volume 10 Issue 4 | 2022 | PP. 23-27