

## The Effect of Ethanol Leaf Extract of *Rauwolfia vomitoria* on Hepatic Markers of Chloroform Intoxicated Albino Wistar Rats

Odom Ruth Obianuju

Department of Biochemistry, Faculty of Science, Ebonyi State University, Abakaliki.

### ABSTRACT

*Rauwolfia vomitoria* is one of the medicinal plants that have served all through the ages as the mainstay in the treatment and preservation of human health due to its medicinal properties. This research was designed to determine the effect of ethanol leaf extract of *Rauwolfia vomitoria* on hepatic markers (ALT, ALP, AST, GGT and bilirubin) of chloroform intoxicated wistar albino rats. All chemicals and reagents used in this study were of analytical grade. The results indicated that ALT, ALP, AST, GGT activities and bilirubin level decreased significantly ( $p < 0.05$ ) in groups 2, 3 and 4 rats treated with graded doses of 100 mg/kg, 200mg/kg and 300mg/kg b.w of ethanol leaf extract of *Rauwolfia vomitoria* when compared with the positive control rats (group 5 ) treated with 0.5 ml of normal saline as shown in figures 3, 4, 5, 6 and 7. Treatment with graded doses of ethanol leaf extract produced significant decrease ( $p < 0.05$ ) in ALT, ALP, AST, GGT activities and bilirubin level compared with the positive control groups. Also there was no significant difference ( $p > 0.05$ ) when (negative control) group 1 rats treated with (0.5 ml of normal saline) were compared with group 4 chloroform intoxicated rats treated with 300 mg/kg b.w. of ethanol extract of *Rauwolfia vomitoria* and group 6 (standard control) rats treated with 5 mg/kg body weight of standard drug Chemiron. In conclusion, the results from this research indicated that ethanol leaf extract of *Rauwolfia vomitoria* decreased liver markers in chloroform intoxicated rats as shown in the treated groups when compared with the untreated control (Positive control). As a result ethanol leaf extract of *Rauwolfia vomitoria* can be used to ameliorate hepatic associated diseases.

Keywords: *Rauwolfia vomitoria*, Hepatic, Markers, Chloroform

### INTRODUCTION

Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs [1,2,3,4,5,6]. They are one of the most important sources of new chemical compounds with potential therapeutic effects [7,8,9]. [10] has advocated traditional medicine as safe remedies for ailments of both microbial and non-microbial origin. Plants are major source of therapeutic compounds and are the essential foundation of medicine since prehistoric time [11,12,13]. Plants synthesize thousands of chemical compounds possessing different properties like defense against insects, bacteria, fungi, diseases and herbivorous mammals [14,15,16]. Herbal and natural products have been used in folk medicine for centuries throughout the world

[17,18,19]. Some Indian medicines like Ayurveda, Sindha and Unani entirely and homeopathy to some extent, depend on plant materials or their derivatives for treating human diseases [20, 21, 22]. Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines [23,24]. Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times [25,26,27]. There has been renewed interest in screening higher plants for novel biologically active compounds, particularly those that effectively intervene in human ailments in the field of chronic diseases [28,29]. Currently, research is focused on the isolation of pharmacologically active compounds from natural sources in the area of those diseases where presently available drugs are not so effective [30]. Also herbal medicines are experiencing

greater resurgence as many people are turning their attention from modern drugs toward parallel herbal systems which are also known as alternative medicine. Plants have been used for centuries as a remedy for human diseases because they possess phytochemicals of therapeutic values [31]. The Indian Traditional medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness [32]. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well documented uses of plant-products is their use as antioxidant

[30]. Hence there is an ever increasing need for health safety in the society filled with toxicants [32]. In spite of tremendous strides in modern medicine, in 2004, the U.S. National Centre for complementary and Alternative Medicine of the National Institutes of Health began funding clinical trials into the effectiveness of herbal medicine [30]. For this reason, various medicinal plants have been studied using modern scientific approaches which have shown that due to various biological components, many of these medicinal plants posse a number of properties such as anti-diabetic, antioxidant, anticancer and anti-inflammatory effects, etc. and can be used to treat a wide range of various diseases [9].

The aim of this research was to determine the effects of ethanol leaf extract of *Rauwolfia vomitoria* on hepatic markers

#### Aim

of chloroform intoxicated wistar albino rats.



Figure 1: The leaves of *Rauwolfia vomitoria*

#### MATERIALS AND METHODS

##### Preparation of the Plant Extract

The leaves of *Rauwolfia vomitoria* were harvested and washed under tap water to remove contaminants and air dried under shade. They were pulverized using laboratory milling machine and sifted

using 0.25 mm sieve. One thousand five hundred gram (1500g) of the powdered leaf sample of *Rauwolfia vomitoria* was soaked in 7500 ml of ethanol for 48 hours with agitation. The resulting methanol

leaf extract was filtered using muslin cloth and evaporated to dryness using rotary evaporator at a temperature of

45°C. The concentrated ethanol leaf extract of *Rauwolfia vomitoria* was used for subsequent analyses.

## Methods

### Preparation of Plant Material

The leaves of *Rauwolfia vomitoria* were collected, dried and milled to powder

using the grinding machine

### Extraction of Plant Material

A known quantity, 500g of ground leaves of *Rauwolfia vomitoria* were macerated in 1500ml of ethanol with thorough shaking at regular interval for 72h at room temperature (26-28°C). The resulting solution was filtered using

Whatman No. 1 filter paper. The filtrates were concentrated using rotary evaporator to obtain slurry of the extract. The semi-pastry extract was stored in the refrigerator and used for the study.

### Determination of Hepatic Markers

Hepatic markers were determined using

standard methods [21].

### Experimental Design

Forty eight (48) Wistar albino rats were used in this study. They were randomly distributed into six (6) groups of 8 rats each. Oxidative stress was induced in the rats and this was performed by intraperitoneal injection of chloroform (100 mg/kg b/w). The rats were fed graded doses of ethanol extract of *Rauwolfia vomitoria* through oral intubation method. The groups and doses administered are summarized below

rats were treated with (100 mg/kg b.w. of ethanol extract of *Rauwolfia vomitoria*).

Group 3: (Chloroform intoxicated rats): rats were treated with (200 mg/kg b.w. of ethanol extract of *Rauwolfia vomitoria*).

Group 4: (Chloroform intoxicated rats): rats were treated with (300 mg/kg b.w. of ethanol extract of *Rauwolfia vomitoria*).

Group 5: (Positive control rats with Chloroform intoxication) were treated with (0.5 ml of normal saline).

Group 1: (Negative control rats without Chloroform intoxication): rats were treated with [0.5 ml of normal saline).

Group 6: (Standard control rats with Chloroform intoxication) were treated with (5 mg/kg bodyweight of standard drug Chemiron).

Group 2: (Chloroform intoxicated rats):

### Statistical Analysis

Results were expressed as mean± standard deviations where applicable. The data were subjected to one-way analysis of variance (ANOVA), followed by Post hoc

Duncan multiple comparison test using SPSS software version 21 and  $p < 0.05$  was regarded as significant.

## RESULTS

The results indicated that ALT, ALP, AST, GGT activities and bilirubin level decreased significantly ( $p < 0.05$ ) in groups 2, 3 and 4 rats treated with graded doses of 100 mg/kg, 200mg/kg and 300mg/kg b.w of ethanol leaf extract of *Rauwolfia vomitoria* compared with the positive control rats (group 5) treated with 0.5 ml of normal saline as shown in figures 2, 3, 4, 5 and 6. Treatment with graded doses of ethanol leaf extract produced significant decrease ( $p < 0.05$ ) in ALT, ALP,

AST, GGT activities and bilirubin level compared with the positive control groups (figures 2, 3, 4, 5 and 6). Also there was no significant difference ( $p > 0.05$ ) when (negative control, group 1) rats treated with (0.5 ml of normal saline) were compared with group 4 rats treated with 300 mg/kg b.w. of ethanol extract of *Rauwolfia vomitoria* and group 6 (standard control) rats treated with 5 mg/kg bodyweight of standard drug Chemiron.

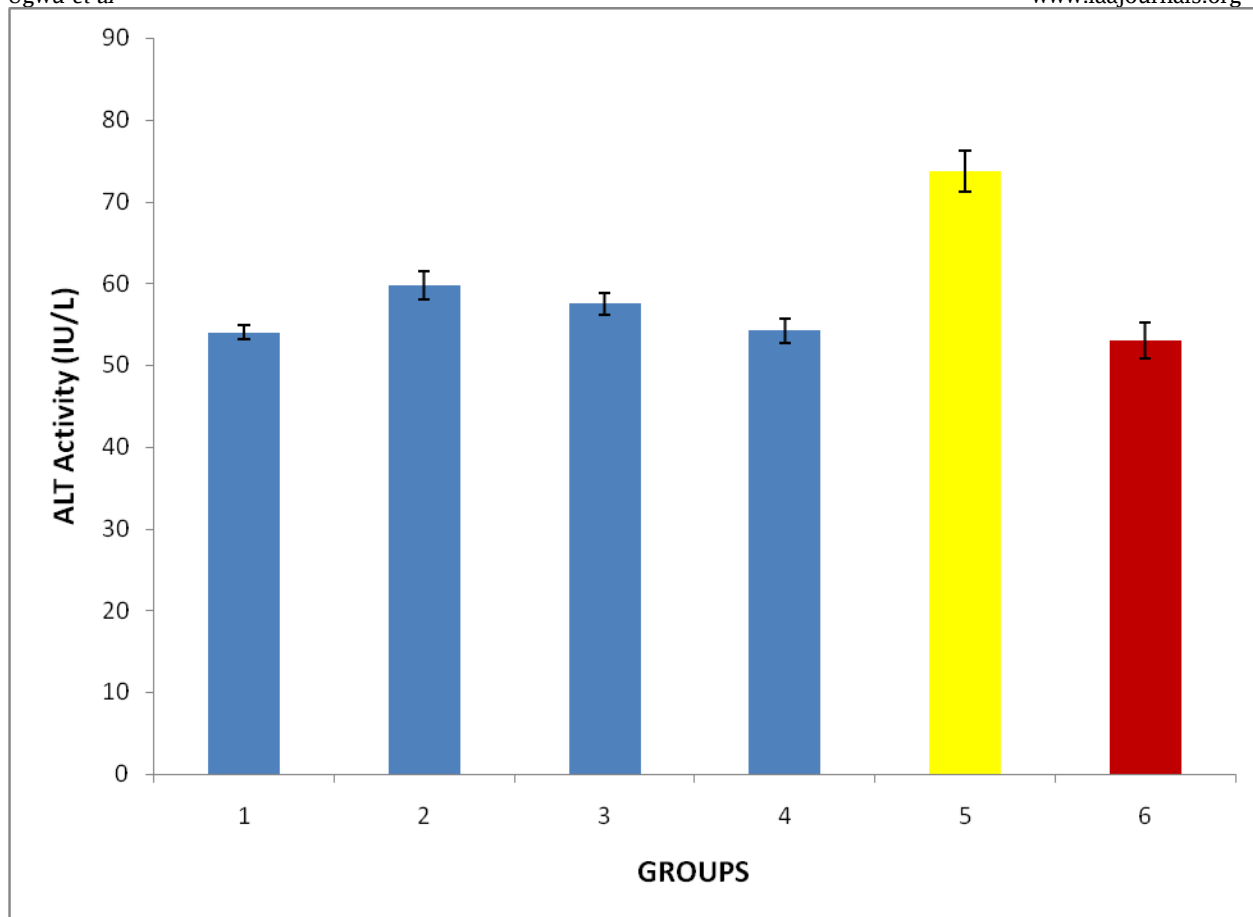


Fig 2: The ALT activity of rats treated with ethanol extract of *Rauwolfia vomitoria*. Data are shown as mean  $\pm$  standard deviation (n=4).

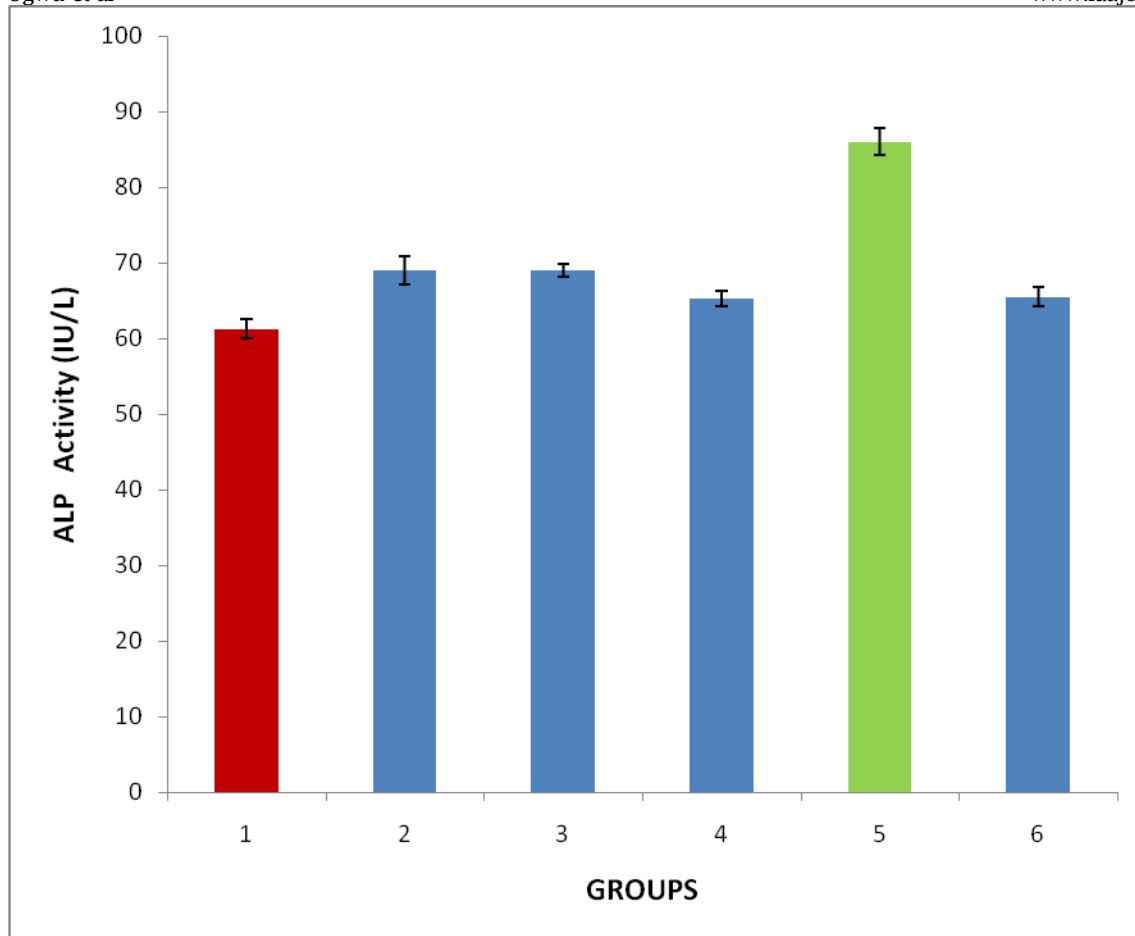


Fig 3: The ALP activity of rats treated with ethanol extract of *Rauwolfia vomitoria*. Data are shown as mean  $\pm$  standard deviation (n=4).

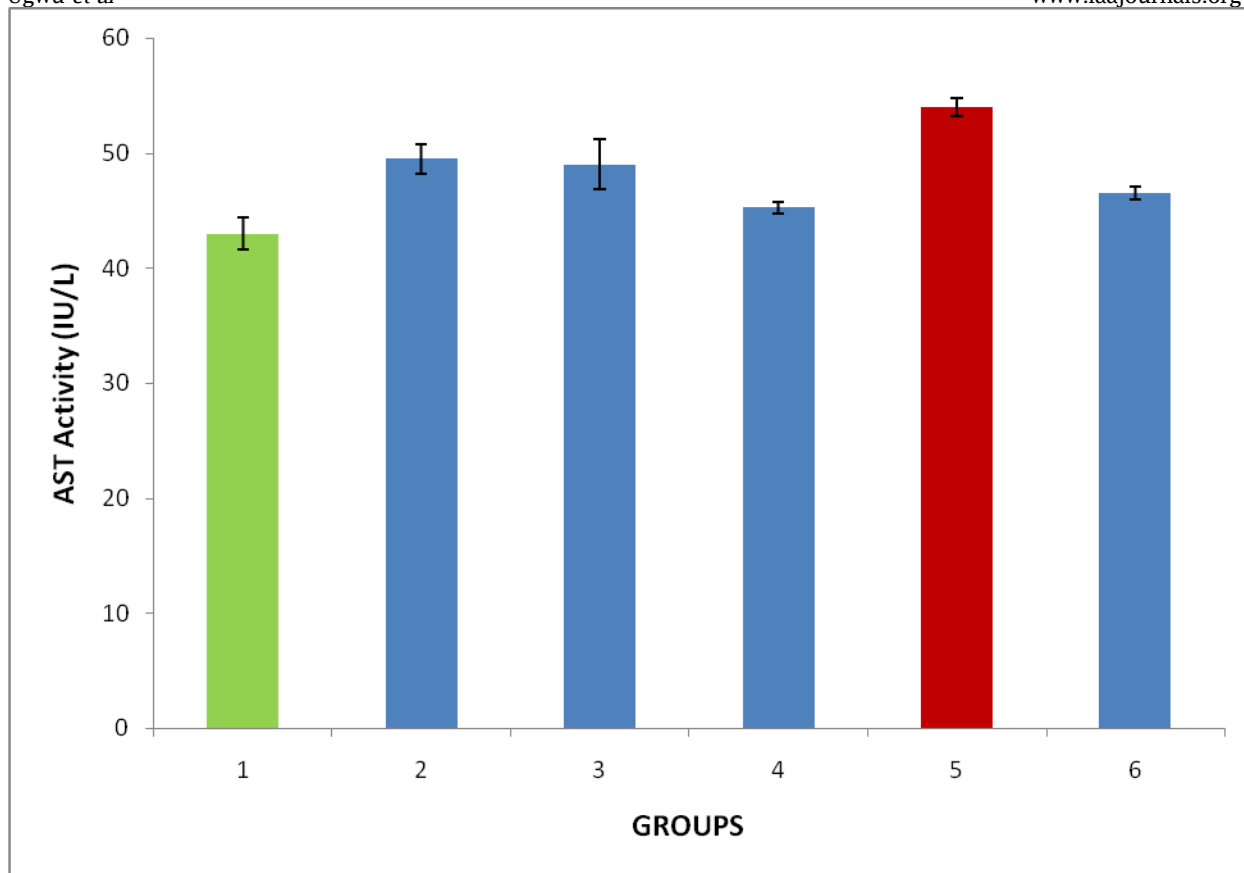


Fig 4: The AST activity of rats treated with ethanol extract of *Rauwolfia vomitoria*. Data are shown as mean  $\pm$  standard deviation (n=4).

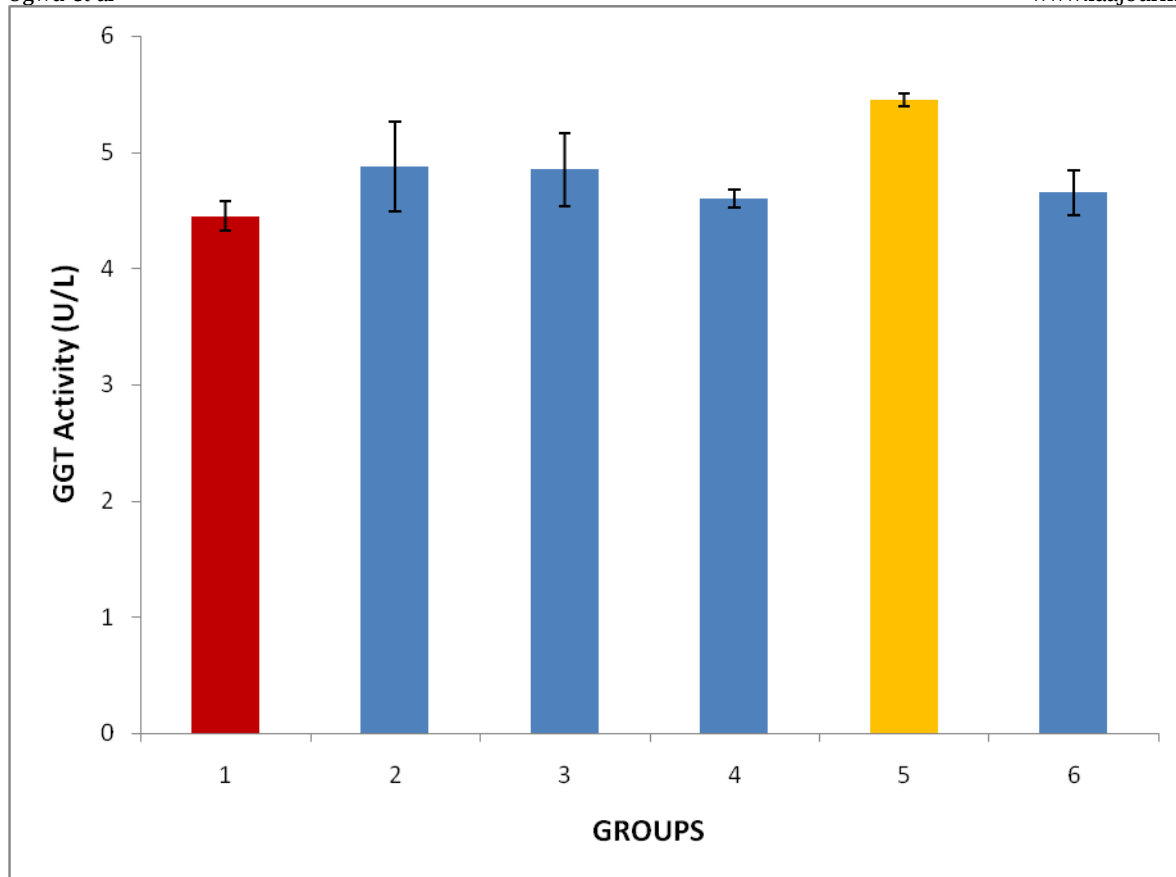


Fig 5: The GGT activity of rats treated with ethanol extract of *Rauwolfia vomitoria*. Data are shown as mean  $\pm$  standard deviation (n=4).

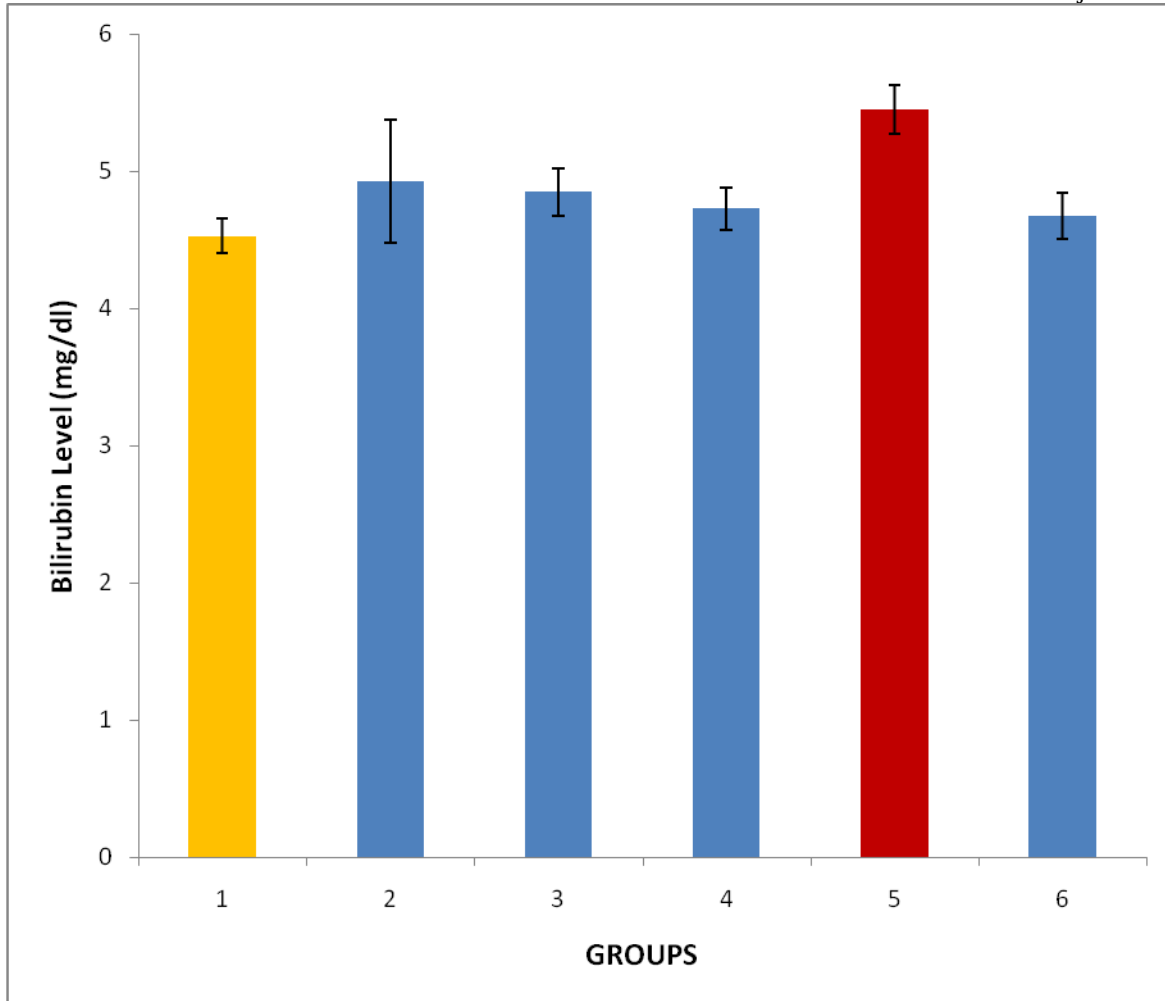


Fig 6: The Bilirubin Level of rats treated with ethanol extract of *Rauwolfia vomitoria*. Data are shown as mean  $\pm$  standard deviation (n=4).

## DISCUSSION

This study was designed to evaluate the ameliorative effect on the liver of chloroform intoxicated Wistar albino rats treated with graded doses of ethanol leaf extract of *Rauwolfia vomitoria*. The results indicated that ALT, ALP, AST, GGT activities and bilirubin level decreased significantly ( $p < 0.05$ ) in groups 2, 3 and 4 rats treated with graded doses of 100 mg/kg, 200mg/kg and 300mg/kg b.w of ethanol leaf extract of *Rauwolfia vomitoria* compared with the positive control rats treated with 0.5 ml of normal saline as shown in figures 3, 4, 5, 6 and 7. Treatment with graded doses of ethanol leaf extract produced significant decrease ( $p < 0.05$ ) in ALT, ALP, AST, GGT activities and bilirubin level compared with the positive control groups (figures 2, 3, 4, 5 and 6). The decreased level in liver markers compared to the known standard drug in group 6 (Standard control) shows

that the extract can be used to ameliorate hepatic damages in liver of chloroform intoxicated rats. This agrees with the work of [21] who obtained similar results on serum amino transferase and alkaline phosphatase activities of rats treated with *Rauwolfia vomitoria* Afzel (Apocynaceae) extract. The liver is the key site of metabolism of xenobiotics and also plays a role in synthesis of drugs. Liver malfunction will impair the function and metabolism of drugs and xenobiotics in the blood [21]. Aminotransferases are cytosolic enzymes widely distributed in tissues with highest concentration in liver and heart but with ALT more specific to the liver and AST to the heart. Damage to the membrane architecture of cells due to exposure to toxicants will lead to their spillage into blood [21].

## CONCLUSION

The ethanol leaf extract of *Rauwolfia vomitoria* decreased the activities of liver markers in serum of the rats when compared with the positive group. This means that hepatic damages can be ameliorated with the used of this medicinal plant. In conclusion, the results from this research indicated that ethanol

leaf extract of *Rauwolfia vomitoria* can decrease liver markers in chloroform intoxicated rats as shown in the treated groups compared with the untreated control (Positive control). As a result ethanol leaf extract of *Rauwolfia vomitoria* can be used to ameliorate hepatic associated diseases.

## REFERENCES

1. Aderemi, F. A. (2004). Effects of replacement of wheat bran with cassava root sieviate supplemented or unsupplemented with enzyme on the haematology and serum biochemistry of pullet chicks. *Tropical Journal of Animal Science*, 7, 147-153.
2. Afolabi, K. D., Akinsoyinu, A. O., Olajide, R., and Akinleye, S. B. (2010). Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake (p.247). Proceedings of 35th Annual Conference of Nigerian Society for Animal Production.
3. Agarwal, S. S. (2001). Development of hepatoprotective formulations from plant sources. In: Pharmacology and Therapeutics in the Millennium. Edited by Gupta SK, Narosa Publishing House, New Delhi, pp: 357-358.
4. Cheng, F., Melissa, B., Fang, X., Shijun, L., Meghan, D., Hongxiu, L., Weizhu, Y., Kenneth, A., Xinxin, D. and Jun, G. (2008). Mechanism of chloroform-induced renal toxicity: Non-involvement of hepatic cytochrome P450-dependent metabolism. *Toxicology and Applied Pharmacology*, 227 (1): Pages 48-55.
5. Cholongitas, E., Shusang, V., Marelli, L., Nair, D., Thomas, M., Patch, D., Burns, A., Sweny, P. and Burroughs, A. K. (2007). Review article: renal function assessment in cirrhosis - difficulties and

- alternative measurements. *Alimentary Pharmacology & Therapeutics*, 26(7):969-78.
6. Corbett, J. V. (2008). Laboratory tests and diagnostic procedures with nursing diagnoses; pp. 90-107.
  7. Dharnidharka, V. R., Kwon, C. and Stevens, G. (2002). Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *American Journal of Kidney Diseases*, 40(2):221-6.
  8. Hawley, G. G. (2015). The Condensed Chemical Dictionary, 10th ed. Van Nostrand Reinhold, New York, NY, p. 237.
  9. Hawley, G. G. (2015). The Condensed Chemical Dictionary, 10th ed. Van Nostrand Reinhold, New York, NY, p. 237.
  10. Hughes, J. and Jefferson, A. (2008). Clinical chemistry made easy. 1st ed. New York, NY: Churchill Livingstone, p. 125-149.
  11. International Programme on Chemical Safety (IPCS) (2017). Chloroform. Environmental Health Criteria 163, WHO: Geneva.
  12. IRIS, Integrated Risk Information System (2001). Toxicological Review of Chloroform. Washington. Environmental Protection Agency.
  13. Isaac, L. J., Abah, G., Akpan, B., and Ekaette, I. U. (2013). Haematological properties of different breeds and sexes of rabbits (p.24-27). Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria.
  14. Johnston, D. E. (2012). Special Considerations in Interpreting Liver Function Tests. *Am Fam Physician*, 59(8):2223-30.
  15. NseAbasi, N. E., Mary, E. W., Uduak, A. and Edem, E. A. (2014). Haematological Parameters and Factors Affecting Their Values. *Agricultural Science*, 2(1):37-47
  16. Ogunbajo, S. O., Alemede, I. C., Adama, J. Y. and Abdullahi, J. (2009). Haematological parameters of Savannah brown does fed varying dietary levels of flamboyant tree seed meal (p. 88-91). Proceedings of 34th Annual Conference of Nigerian Society for Animal roduction.
  17. Olafedehan, C. O., Obun, A. M., Yusuf, M. K., Adewumi, O. O., Oladefedehan, A. O., Awofolaji, A. O. and Adeniji, A. A. (2010). Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits (p.212). Proceedings of 35th Annual Conference of Nigerian Society for Animal Production.
  18. Österreicher, C. H. and Trauner, M. (2012). Xenobiotic-induced liver injury and fibrosis. *Expert Opinion on Drug Metabolism and Toxicology*, 8(5): 571-80.
  19. Owolabi, O. J., Amaechina, F. C. and Okoro, M. (2011). Effect of ethanol leaf extract of *NewbouldiaLaevison* blood glucose levels of diabetic rats. *Tropical Journal of Pharmaceutical Research*, 10(3): 249-254.
  20. Prajapati, N. D., Purohit, S. S., Sharma, A. K., and Kumar, T. (2003). A hand book of medicinal plants. *Agribios*, p553.
  21. Pratt, D. S. and Kaplan, M. M. (2000). Evaluation of Abnormal Liver Enzyme Results in Asymptomatic Patients. *NEJM*, 342(17):1266-71.
  22. Priem, F., Althaus, H., Jung, K. and Sinha, P. (2011). Beta-trace protein is not better than cystatin C as an indicator of reduced glomerular filtration rate. *Clinical Chemistry*, 47(12):2181.
  23. Pucci, L., Triscornia, S., Lucchesi, D., Fotino, C., Pellegrini, G., Pardini, E., Miccoli, R., Del, P. and Penno, G. (2007). Cystatin C and estimates of renal function: searching for a better measure of kidney function in diabetic

- patients. *Clinical Chemistry*,53(3):480-8.
24. Randall, J. R., James, E. K., Norman, E. S., Augusta, B. A., David, A. L., Michael, A. P. and Peter, J. G. (2012). Mechanisms of Chloroform and Carbon Tetrachloride Toxicity in Primary Cultured Mouse Hepatocytes. *Environmental Health Perspectives*, 69, 301-305.
25. Randall, J. R., James, E. K., Norman, E. S., Augusta, B. A., David, A. L., Michael, A. P. and Peter, J. G. (2012). Mechanisms of Chloroform and Carbon Tetrachloride Toxicity in Primary Cultured Mouse Hepatocytes. *Environmental Health Perspectives*, 69, 301-305.
26. Randers, E., and Erlandsen, E. J. (2016). Serum cystatin C as an endogenous marker of the renal function--a review. *Clinical Chemistry and Laboratory Medicine*,37(4):389-95.
27. Rosner, M. H. and Bolton, W. K. (2006). Renal function testing. *American Journal of Kidney Diseases*,47(1):174-83.
28. Shivaraj, G., Prakash, B. D., Shruthi, S. K., Vinayak, V. H., Avinash, A. K. and Sonal, N. V. (2010). Markers of renal function tests. *North American Journal of Medical Sciences*,2(4): 170-173.
29. Shlipak, M. G., Katz, R., Fried, L. F., Jenny, N. S., Stehman-Breen, C., Newman, A. B., Siscovick, D., Psaty, B. M. and Sarnak, M. J. (2005). Cystatin-C and mortality in elderly persons with heart failure. *Journal of the American College of Cardiology*,45(2):268-71.
30. Togun, V. A., Oseni, B. S. A., Ogundipe, J. A., Arewa, T. R., Hammed, A. A., Ajonijebu, D. C. and Mustapha, F. (2007). Effects of chronic lead administration on the haematological parameters of rabbits - a preliminary study (p. 341). Proceedings of the 41st Conferences of the Agricultural Society of Nigeria.
31. U.S. Environmental Protection Agency (U.S. EPA) (2011). Toxicological Review of Chloroform.
32. Ugwu, O. P. C., Edwin, N. and Ogbanshi, M. E. (2015). The Effect of Ethanol Leaf Extract of *Jatropha curcas* on Chloroform Induced Hepatotoxicity in Albino Rats. *Global Journal of Biotechnology & Biochemistry*10 (1): 11-15.