# Histological Study of the Anti-Oxidative Potentials of Ethanolic Leaf Extract of *Talinum Triangulare* (Water Leaf) on the Liver of Monosodium Glutamate (MSG) Induced Hepatotoxicity in Albino Wistar Rats

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**ABSTRACT** Monosodium glutamate (MSG) is a widely used flavour enhancer despite its documented adverse effect on humans and animals. *Talinum triangulare* (water leaf) is one of the commonest vegetables known for its nutritious and medicinal properties. This study was therefore designed to investigate the anti-oxidative potentials of ethanolic extract of *Talinum triangulare* on the liver of monosodium glutamate induced hepatoxicity. Twenty-five male wistar rats weighing 100-150mg were used for the study. They were acclimatized under standard laboratory conditions for two weeks and randomly divided into 5 groups designated A, B, C, D and E with five rats each. Group A served as control and was given 1ml of distilled water, group B received 2000mg/kg of MSG, group C and D received 100mg/kg and 200mg/kg of ethanolic leaf extract of *Talinum triangulare* plus 2000mg/kg MSG respectively while group E received 100mg/kg of ethanolic extract of *Talinum triangulare*. All administrations were done orally for 21 days. All animals were sacrificed under ketamine anaesthesia (4mg/kg i.p.), blood was collected via cilliary puncture for estimation of serum AST, ALP and ALT. The liver was harvested and processed for routine H&E staining. Histological study of the liver revealed normal histological features - central vein, hepatocytes and cords of hepatocytes, group B showed congested central vein, disorganisation of hepatic cords and necrosis of hepatocytes while groups C, D and E showed normal histology. There was significant increase in serum AST levels in groups B, C, D and E compared to control. There was no significant difference in ALT and ALP levels in all groups. These results revealed that *Talinum triangulare* attenuated the effects of MSG on the liver

**KEYWORDS** monosodium glutamate, *Talinum triangulare*, liver, hepatotoxicity

## INTRODUCTION

Monosodium glutamate (MSG) known as AJI-NOMOTO is the sodium salt of glutamic acid (Eweke, 2007). MSG contains 78% of glutamic acid, 22% of sodium and water (Samuel, 1998). Glutamate is one of the most common amino acids found in nature and is the main component of many proteins and peptides of most tissues. Glutamate is also produced in the body and plays an essential role in human metabolism. It is a major component of many protein-rich food products either in free or bound state in animal products such as meat, fish, milk and cheese or vegetable origins such as mushroom and tomato (IFIC, 1994). MSG is used as a flavor enhancer, it provides a flavoring function similar to the naturally occurring free glutamate which differs from the four classic tastes of sweet, sour, salty and bitter (Leung and Foster, 1996). As food additive, MSG is described and listed on food labels as a “Flavoring” or “hydrolyzed vegetable protein”. Through its stimulation of the orosensory receptors and by improving the palatability of meals, MSG influences the appetite positively, and induces weight gain. Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to human and experimental animals (Biodun *et al.*, 1993). MSG could produce

symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches. In addition to these MSG symptom complex, ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, atopic dermatitis, ventricular arrhythmia, neuropathy, abdominal discomfort and adverse effect on hepatic functions which might be due to oxidative stress induced by MSG (Geha *et al.*, 2001). Despite the health problems seemingly associated with the use of MSG, reputable International Organizations and Nutritionists have continued to endorse MSG, reiterating that it has no adverse reactions on humans. Notably of such is the directorate and regulatory affairs of Food and Drug Administration and Control (FDA&C) in Nigeria, now NAFDAC.

NAFDAC has certified MSG as a safe and wholesome product that is not injurious to health (Okwaraiwe, 1992).

Waterleaf (*Talinum triangulare*) is predominantly one of the commonest nutritious annual vegetables; a smooth herbaceous plant found in most Nigerian markets, it has succulent leaf and stem that are edible. It has also been shown to have therapeutic potentials in traditional medicine due to its nutritional supplement with minimal level of oxalic acid compared to some other plants supplements with severe adverse effect (Ajah *et al.*, 2010). Waterleaf is rich in Vitamin A and C, omegs-3- fatty acids and minerals such as iron and calcium (Ezekwe *et al.*, 2001). Waterleaf is also rich in crude protein (22.1%), crude fiber (11.12%), and ash (33.98%). All these vitamins and minerals contribute to high anti-oxidant values of waterleaf. (Enete and Okon, 2010). Waterleaf has medicinal properties which is beneficial in enhancing cerebral functioning, regulating blood sugar levels as well as cholesterol levels. It aids easy digestion, boost blood level, and helps in the treatment of hepatic aliment (Ezekwe *et al.*, 2013).

Liver is a vital organ of vertebrates and some other animals (Maton *et al.*, 1993). It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, plasma protein synthesis, production of bile; an alkaline compound which aids in digestion and detoxification of most substances (Gartner and Hiatt, 2000). Since the liver is involved in the performance of these varied functions, it may be susceptible to injury resulting from toxic substances.

## MATERIALS AND METHOD

### *Procurement and Preparation of Extract of Talium Triangulare*

The water leaves used for this study were bought from a local market in Ihiala, Ihiala Local Government, Anambra State. The leaves were destalked, washed and air dry for 15 days. The water leaves were grounded using laboratory mill into coarse form. 250mg of the grounded leaves were macerated in 100mls of 98% absolute ethanol (BDH) and was allowed to stay for 24 hours after which it was sieved using porcelain cloth and was further filtered using Whatmann filter paper into a clean glass beaker, the filtrate was concentrated using Digital Rotary Evaporator (TT-53 Techmel and Techmel, USA) and was further dried using Thermostat Oven (DHG-9023A PECmedicals USA) into a gel-like substance and was stored in a Refrigerator (Nexus) for further use.

### *Experimental Design*

Twenty-five wistar rats were used for the study, they were randomly divided into five groups – A, B, C, D and E with five rats in each group. ` Group A served as control while B, C, D and E served as experimental groups. They were acclimatized for two weeks. Administration followed acclimatization for 21 days. All groups received feed (growers mesh) and water *ad libitum.*

- Group A: (control group) received 1ml of distilled water
- Group B, received 2000mg/kg body weight of MSG.
- Group C and D, received 100mg/kg and 200mg/kg body weight of *Talinum triangulare* extract respectively plus 2000mg/kg body weight of MSG.
- Group E: received only 100mg/kg body weight of *Talinum Triangulare* extract

All administrations were done orally using orogastric tube.

### *C. Termination of Experiment*

On the 22nd day of the experiment, the animals were anesthetized and blood samples were collected via ciliary puncture for serum estimation of ALP, AST and ALT. The liver was harvested and fixed for histological processing.

## RESULTS

### *Histological Results*

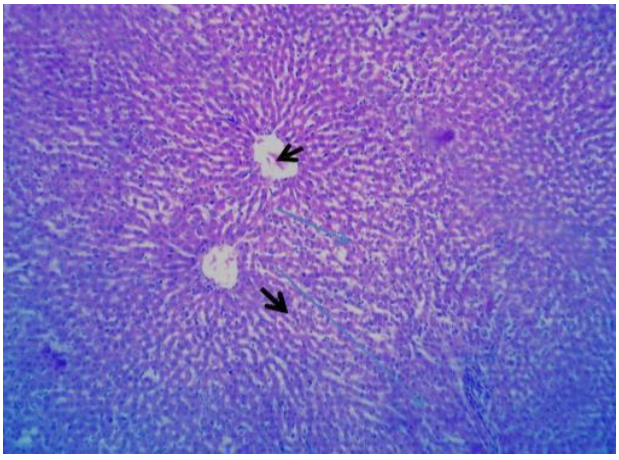

**FIGURE 1. Representative photomicrograph of section of the liver of control group showing the central vein, cords of hepatocytes well preserved, and sinusoids (Hematoxylin and eosin method) mag X100**

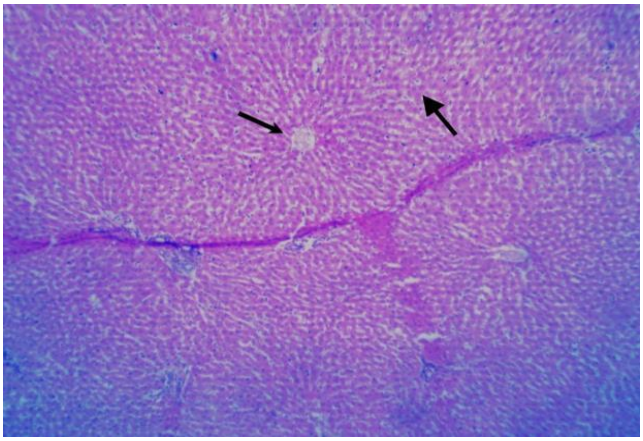

**FIGURE 2. Representative photomicrograph of liver treated with 2000mg/kg body weight of MSG showing congestion central vein, disorganization of hepatic cords, and necrosis of hepatocytes (Hematoxylin and eosin method) mag X100**

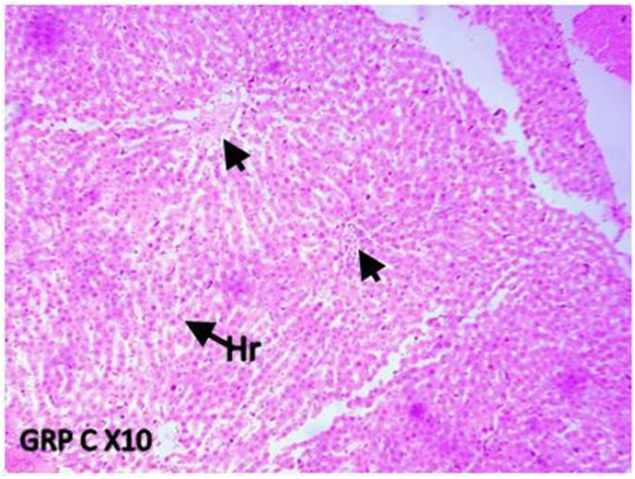


**FIGURE 3. Representative photomicrograph of the liver treated with 100mg/kg body weight of ethanolic extract of *Talinum triangulare* and 2000mg/kg body weight of MSG showing mild congested central vein (black arrow), regeneration of hepatic cells (Hr). (Hematoxylin and eosin method) mag X100**

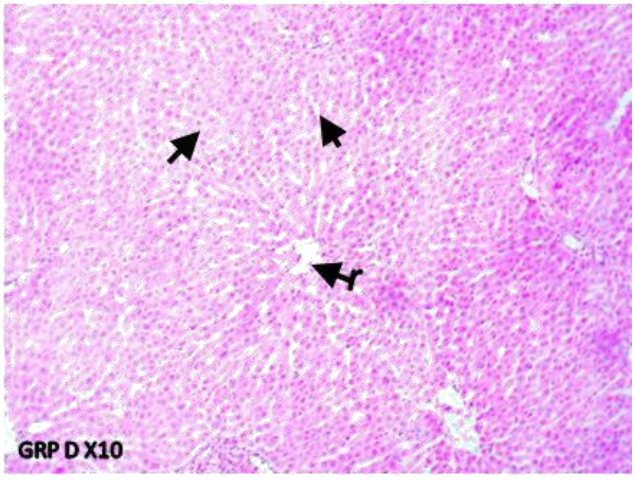


**FIGURE 4. Representative photomicrograph of the liver treated with 200mg/kg body weight of ethanolic extract of *Talinum triangulare* and 2000mg/kg body weight of MSG showing restored central vein (*r*), regeneration of hepatic cells with no vacuolated sinusoids. (Hematoxylin and eosin method) mag X100**

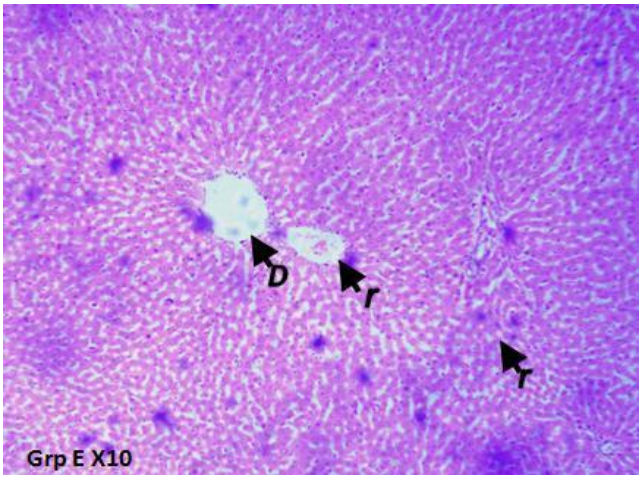


**FIGURE 5. Representative photomicrograph of the liver treated with 100mg/kg body weight of ethanolic extract of *Talinum triangulare* showing normal central vein (*r*), normal hepatic cells with no vacuolated sinusoids. (Hematoxylin and eosin method) mag X100**

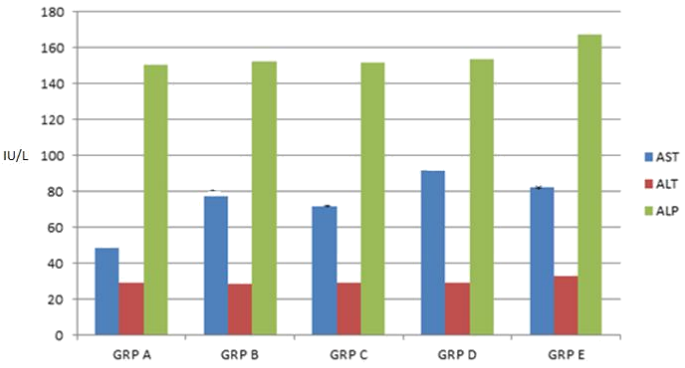


**FIGURE 6. Graph showing serum levels of AST, ALT, and ALP in U/L**

### *Estimation of serum AST, ALT, and ALP Levels*

**TABLE 1. The effect of Ethanolic extract of *Talinum triangulare* and MSG, *and Talinum triangulare* on Aspartate Transaminase, Alanine Transaminase, and Alkaline Phosphatase level after 21 days of treatment.**

| | | MEAN ±SEM | | P-VALUE | F-VALUE |
|---|---|---|---|---|---|
| Aspartate Transaminase (U/L) | Group A (Control) | 48.66 | ±0.33 | | |
| | Group B (2000mg/kg of MSG) | 77.33 | ±0.33 | 0.000* | 324.102 |
| | Group C (100mg/kg of T. T +2000mg/kg of MSG) | 71.67 | ±1.67 | 0.000* | |
| | Group D (200mg/kg of T. T+2000mg/kg of MSG) | 92.00 | ±1.00 | 0.000* | |
| | Group E (100mg/kg only of T.T) | 82.00 | ±1.23 | 0.025 | |
| Alanine Transaminase (IU/L) | Group A (Control) | 29.00 | ±0.33 | | |
| | Group B (2000mg/kg of MSG) | 28.67 | ±0.00 | 0.580 | 0.611 |
| | Group C (100mg/kg of T. T +2000mg/kg of MSG) | 29.33 | ±0.66 | 0.282 | |
| | Group D (200mg/kg of T. T+100mg/kg of MSG) | 29.08 | ±0.33 | 1.000 | |
| | Group E (100mg/kg only of T.T) | 32.90± | 0.18 | 0.125 | |
| Alkaline Phosphatase (IU/L) | Group A (Control) | 150.67 | ±1.37 | | |
| | Group B (2000mg/kg of MSG) | 152.66 | ±2.00 | 0.506 | 1.106 |
| | Group C (100mg/kg of T. T +2000mg/kg of MSG) | 151.67 | ±0.67 | 1.000 | |
| | Group D (200mg/kg of T. T+2000mg/kg of MSG) | 153.67 | ±0.33 | 0.156 | |
| | Group E (100mg/kg only of T.T) | 167.34 | ±0.87 | 2.034 | |

**All data were analyzed using One-way Anova, followed by multiple comparism using LSD, and data were considered significant at P<0.05. *P<0.05 shows that it was significant, **P<0.05 shows that it was more significant and P> 0.05 means not significant.**

There was a significant increase (P<0.05) in AST level in groups B, C, D, and E when compared to group A. ALT result showed a non-significant increase (P>0.05) in group C, D, and E and a non-significant decrease in group B. Result for ALP showed a non-significant increase (P<0.05) in AST level in group B, C, D and E when compared to group A.

## DISCUSSION

Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds that are for biological functions, including defense against insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. (Igbayilola *et al.*, 2017). Geha *et al* (2000) mentioned that monosodium glutamate (MSG), a flavor enhancer and food addictive is recognized as a neurotoxin.

Congestion of central vein, disorganisation of hepatic cords, and necrosis of hepatic cells observed in group B, that received 2000mg/kg body weight of MSG may be as a result of oxidative stress induced by MSG. This result was also reported by Eweka *et al.,* (2011) and Ogbuagu *et al.,* (2017).

Hepatic vacuolation is an indication of impairment in triglyceride secretion and decrease in carrier lipoprotein synthesis. Congestion of the central veins is associated with a change in the amount of fatty acids. As observed in the study, MSG caused congestion of the central vein. This observation was also reported by Hanaa and saleh (2006) who carried out an investigation on the Effect of MSG on Rat Liver and the Ameliorating effect of Guanidine Ethane Sulfonic Acid.

The vitamins and minerals present in waterleaf contribute to its high antioxidant properties. and antioxidants protect the body cells against the damaging adverse effects of reactive oxygen species. The antioxidants contained in water leaf attenuated the effect of MSG on the liver in a dose dependent manne.

The biochemical analysis of liver enzymes showed a significant increase in the AST levels of group B, C, D, and E, and a non-significant difference in the ALT and ALP levels. These tests are non-specific (Monica, 2010) and hence does not necessarily indicate liver dysfunction. The increase in AST levels of all groups compared to group A could be from cellular injury from other body tissues where AST is also found in a large amount compared to the level found in liver.

## CONCLUSION

The results of the present investigation have shown that monosodium glutamate (MSG) is capable of producing alterations in the liver and that the anti-oxidative properties of waterleaf ameliorated hepatotoxicity in a dose related manner via inhibiting oxidative stress.

## REFERENCES


1. Ayeni, F.A., Alawode, D., Joseph, D., Sukop, P., Olawuyi, V., and Emmanuel T. (2014). Investigation of Talinum Triangulare Plant Extract as Corrosion Inhibitor for Aluminium-Copper-Magnesium Alloy in Acidic Medium. Journal of Minerals and Materials Characterization and Engineering. 2: 286-291.
2. Aja, P.M., Okaka, A.N., and Onu, P.N.A. (2010). Phytochemical Composition of Talinum triangulare (Water Leaf) Leaves. Pak Journal of nutrition 9:527-530.
3. Andarwulan, N., Batar, R., Sandrasari, D. A., Bolling, B., and Wijaya, H. (2010). Flavonoid content and antioxidant activity of vegetables from Indonesia. Food Chemical 121:1231-1235.
4. Bahar, E., Ara, J., Alam, M., Nath, B., Bhowmik, U., and Runi, N. (2013). In-vitro antioxidant and thrombolytic activity of ethanol extract of water leaf. Journal of Pharmacognosy and Phytochemistry. 2(2): 125-133.
5. Bancroft, J.D., and Gamble, M. (2008). Theory and Practice of Histology Techniques, 6th edition. Churchill Livingstone Elsevier, Philadelphia. 83 - 134.
6. Biodun, B., and Biodun, A. (1993). "A Spice or Poison? Is Monosodium Glutamate Safe for Human Consumption?" National Concord Journal p.34.
7. Enete, A.A and Okon, U.E. (2010). Economics of Waterleaf production in Akwa Ibom State, Nigeria. Field Actions Science reports [Online], Vol 4 page 1-5.
8. Eweka, A.O (2008). Histological studies of the effects of MSG on the liver of adult wistar rats. The international journal of Gastroenterology.
9. Eweka, A. O. (2007). "Histological Studies of the Effects of Monosodium Glutamate on the Kidney of Adult Wistar Rats," The International Journal of Health, Vol. 6, No. 2. Page 46
10. Eweka, A.O. (2010). Histological studies of the effects of monosodium glutamate of the fallopian tubes of adult female Wistar rats. North American Journal of Medical Science 2:146-149.
11. Ezekwe, C.I., Chidinma, R.U., and Okechukwu, P.C.U. (2013). The effect of Methamolic Extract of Waterleaf on the Hematology and some liver parameters of Experimental Rats. Global Journal of Biotechnology and Biochemistry (2): page 51-60.
12. Ezekwe, M.O., Besong, S.A., and Igbokwe, P.E. (2001). Beneficial influence of Pursiane and Waterleaf supplement to human. Federation of American Societies for Experimental Biology Journal 16: A639.
13. Gartner, L.P and Hiatt, J.L. (2000). Color Atlas of Histology 3rd Edition New York:
14. Lippincott Williams and Wilkins Publishers. Page 294-301.
15. Geha, R.S., Beiser, A., Ren, C., Patterson, R., Grammar, L.C., Ditto, A.M., and Harris, E.K. (2001). Review of Allergic Reaction to Monosodium Glutamate and Outcome of a Multicenter Double-Blind Placebo-Controlled Study. Journal of Nutrition, Vol. 130, page 1032S-1038
16. Hanaa, F.W., and Saleh, E. (2006). The Effect of Monosodium Glutamate (MSG) On Rat Liver and The Ameliorating Effect of "Guanidine Ethane Sulfonic acid (GES). The Egyptian Journal of Hospital Medicine. Volume 4. Page 524-538.
17. Igbayiilola, Y.D., Morakinyo, A.O., Ewetayo, A.W., Oyabami, A.O., and Saka, W.A. (2017). Biochemical and Antioxidant effects of Waterleaf in Female Sprague-Dawley rats. International Journal of Basic and Applied Physiology. 6(1): 22-28.
18. Investment Funds Institute of Canada (IFIC). (1994). Review of Monosodium Glutamate, Examining the Myths.
19. Keeffe, E.B., and Friedman, L.M. (2004). Handbook of liver disease. Page 104-123.
20. Leung, A., and Foster, S. (1996). Encyclopedia of Common Natural Ingredient Used in Food, Drugs and Cosmetics, New York. Page 373-375.
21. Leung, W.T., Busson, F., and Jardin, C. (1963). Phytochemical composition of Talinum triangulare and food composition table for use in Africa. FAO, Rome, Italy. Page 306.
22. Maton, A., Jean, H., Charles, W.M., Susan, J., Maryanna, Q.W., and David, L. (1993). Human Biology and Health. Englewood Cliffs, New Jersey, USA, Prentice Hall, ISBN 0-13-981176-1.
23. Monica, C (2010). Textbook of District Laboratory Practice in Tropical Countries. Second edition. Page 171-184.
24. Nakanishi, Y., Tsuneyama, K., Fujimoto, M., Salunga, T.L., Nomoto, K., An, J.L., and Shimada, T. (2008). Monosodium Glutamate (MSG): A Villain and Promoter of Liver Inflammation and Dysplasia. Journal of Autoimmunity. (1) page 42-50.
25. Nehal, F.E., Doaa, A.R., Maysa, F.S., and Manal E.E. (2017). Effect of Monosodium Glutamate on Body Weight and Histological Structure of the Zona Fasciculata of the Adrenal Cortex in Young Male Albino Rats. Tanta Medical Journal. Volume 45. Issue 2. Page 104-113
26. Okwurawe, P.E. (1992). The role of Food and Drug Administration and control (FDA&C) in ensuring the role of food and food ingredients; A symposium held at Sheraton Hotel, Lagos.
27. Samuel, A. (1999). The Toxicity/Safety of MSG: A Study in Suppression of Information Accountability in Research, Vol. 6, No. 4 page 259-310.
28. Tawfik, S., and Al-Badr. (2012). Adverse Effects of Monosodium Glutamate on Liver and Kidney Functions in Adult Rats and Potential Protective Effect of Vitamin C and E. Journal of Food and Nutrition Sciences. Vol (03): page 651-659.