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Evaluating the curative and protecting role of graviola leaf extract against hepatic and hematological disorders caused by monosodium glutamate in male rats

Wafaa M. EL-Kholy, Mustafa Sh. Atta and Walaa M. Nassar

Physiology Division, Zoology Department, Faculty of Science,

Mansoura University, Mansoura, Egypt

Corresponding author: E-mail: walaanassar2010@gmail.com

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Abstract: The objective of the current study was to establish the curative and protective function of Graviola Leaf Extract versus MSG-produced hepatic groups, six for each as follows: Group 1(control): rats receiving distilled water; group 2 (G): and hematological disorders in Male Wistar Rats. Rats were haphazardly divided into five rats receiving G (200 mg / kg bw) regular oral administration for one month; group 3 (MSG): rats receiving MSG (100 mg / kg bw) daily oral administration for one month; group 4 (G+MSG): rats receiving G oral administration for one month and MSG for another month; group 5 (MSG+G): rats are given MSG orally for one month, followed by G for another month. Our results showed significant increases in body weight, TC, TG, LDL-C, AST, ALT, ALP, GGT, TB in serum as well as AST, ALT, ALP, GGT in liver tissue as well as major decreases in HDL-C, TP, Alb and improvement in CBC parameters such as (RBCs, Hb, Hct percent, MCH, MCV, WBCs). On the other hand, G administration significantly improved the deviation resulting from MSG in all parameters and marked CBC development. Ultimately, it could be concluded that to reduce the hepatic and hematological from damage caused by MSG and G should be taken before MSG as a protective agent **.**

keywords: Graviola, MSG, Hepatotoxicity, Hematological parameters **.**

Abbreviations: G, graviola; MSG, monosodium glutamate; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TB, total bilirubin; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; TP, total protein; Alb, albumin, CBC, complete blood count.

1.Introduction

(Monosodium glutamate (MSG) is one of the most common amino acids found in nature, the sodium salt of glutamic acid (non-essential amino acids). MSG contains 78% glutamic acid, 22% sodium and water [1]. It is used as a flavor enhancer [2].

Graviola is an evergreen tree, is a medicinal plant, It is native to South and North America's warmest tropical areas **[3].** Also the plant has shown to have many Phytoconstituents and compounds such as: alkaloids (alks), megastigmans (mgs), flavonol triglycosides (FTGs), phenolics (PLs), cyclopeptides (Cps) and essential oils, various important minerals such as K, Ca, Na, Cu, Fe and Mg and annonaceous acetogenin compounds (AGEs) which is the major component in graviola [4].

2. Materials and methods

MSG salt purchased from Metro town market, Egypt, Monosodium glutamate liquified in dist. H_2O And was given orally at a dosage (100 mg/kg bw) to male albino rats Graviola Dry Extract was purchased from Original Natural Company (Quarrata, Pistoia, Italy) (product code:912943735. All other chemicals were purchased from generic verified firms.

Animals: Thirty male albino rats (*Rattus norvegicus*) 90-100g used for this study. Rats

were purchased from Egyptian Institute for Vaccine and Serological manufacture, Helwan, Egypt and were housed in the animal house of the Department of Physiology, Faculty of Veterinary, Kafer-Elsheikh University. Rats located in stainless steel cages containing wood-chip for bedding and renewed daily. The rats maintained a 24 h cycle in a controlled temperature setting. Two weeks before the start of the experiment, all rats were acclimatized to the location. During the experiment time, the animals were provided with normal diet and water *ad libitum*

Animal grouping

After two weeks of acclimatization period, Animals were divided randomly into five groups, each consisting of six animals as follows: Control group: in this group, rats received distilled water. Graviola (G) treated group: rats were given G (200mg/kg bw) per gavage orally daily for 4 wks. Monosodium glutamate (MSG) treated group: MSG (100mg/kg bw) was given orally for 4 wks daily. Graviola and Monosodium glutamate (G+MSG) treated group: rats were given orally G only (200 mg/kg bw) daily for 4 weeks, followed by MSG (100 mg / kg bw) for another 4 wks. Monosodium glutamate and graviola (MSG+G) treated group: rats were given MSG orally only (100 mg / kg bw) per day for four weeks, followed by G (200 mg / kg bw) per day for another four weeks.

Sample collection:

Overnight fasted rats were sacrificed (24 hours after the last treatment) and blood samples were collected and divided into two parts in clean glass centrifuge tubes, the first part was left to clot and then centrifuged for 15 minutes at 4000 rpm, and the second one blood sample were collected in sterilized EDTA tubes for complete blood count (CBC) analysis.

The clear non hemolyzed sera were quickly removed and put in labeled Eppendorf's tubes; the sera were frozen at -20^{o}C for different biochemical analysis.

Used kits:

TC, TG, HDL-C, ALP,GGT were estimated according to the method of [5-9] using TC Biodiagnostic kit from Biodiagnostic Co. Dokki, Giza, Egypt.LDL-C concentration was calculated according to equation described by [10].

ALT and AST in serum and liver were measured according to the colorimetric kit technique using RAM diagnostic kit [11]. ALP and GGT activities in serum and liver were measured according to [8, 9], respectively, TP and Alb in serum were measured according to the method of [12, 13] respectively.Total bilirubin level in the serum was determined using a kit purchased from Diamond diagnostics, Co. Cairo, Egypt, measured by a colorimetric method of [14]. Hematological parameters were measured using Haematological Analyzer Swelab Alfa Sweden part No 1400016.

Statistical analysis:

Using GraphPad Prism 5.0, data are analyzed. The findings of the experiment were expressed as mean ± standard error mean (SEM) (n= 6). Results was analyzed by a one-way analysis of variance (ANOVA) followed by multiple correlation testing by Newman–Keuls. Values deemed statistically significant for $P \leq 0.05$.

Results:

Oral administration with MSG caused marked increase in body weight and serum lipid profile as (TC, TG and LDL-C) and marked reduction in HDL-C as well as marked upsurge in serum liver enzymes as (ALT, AST, ALP and GGT), TB and significant decrease in TP and Alb content as well as MSG affected the hematological parameters as (Hb, MCH, RBCs, MCV, Hct%, WBCs, lymphocyte, neutrophil and platelets) as shown in the following tables. Results are presented as means ± SE for 6 rats in each group, similar letters (non-significant), Different letters (significant), change at $P \leq 0.05$. C: control group, G: Graviola, MSG: Monosodium glutamate., PLTs: platelets

Table (1): Mean of body weight changes (g) in all studied groups**.**

Time/day	C	G	MSG	G+MSG	MSG+G
Zero day	179.9±1.23	181.1±1.50	178.8±0.99	178.3±0.98	178.1±1.oo
1st ten days	202.0±2.70	197.3±1.68	210.4±1.85	195.6±2.99	193.9±2.60
2nd ten days	217.4±2.38	212.3±2.52	230.8±2.76	209.2±2.60	208.3±1.75
3rd ten days	228.7±2.42	218.3±3.00	240.6±3.35	213.0±1.84	212.1±3.68
4th ten days	245.0±1.83	236.7±2.10	267.3±3.11	229.8±2.88	224.1±4.19
5th ten days	259.7±2.61	250.7±2.32	280.9±2.83	241.7±2.59	237.2±3.03
Final day	276.6±3.07	269.6±2.85	305.7±3.24	246.9±2.13	245.3±2.08

Results are presented as means ± SE for 6 rats in each group, similar letters (non-significant), Different letters C: control group, G: graviola, MSG: monosodium glutamate

Table (2): Outcome on lipid profile in serum of rat.

Parameters	C	G	MSG	G+MSG	MSG+G
TC (mg/dl)	108.10^{a}±0.74	106.04^{a}±0.75	125.50^{b}±0.71	118.00^{c}±0.74	119.20^{c}±0.77
TG(mg/dl)	97.76^{a}±0.70	95.33^{a}±0.71	130.00^{b}±0.74	114.80^{c}±0.74	117.80^{c}±0.77
HDL-C(mg/dl)	50.40^{a}±0.74	51.35^{a}±0.62	30.81^{b}±0.74	44.79^{c}±0.74	42.43^{c}±0.74
LDL-C (mg/dl)	33.92^{a}±0.84	32.81^{a}±0.72	72.80^{b}±0.74	56.61^{c}±0.70	55.40^{c}±0.74

Results are presented as means ± SE for 6 rats in each group, similar letters (non-significant), Different letters (significant), P≤0.05. **C**: control group, **G**: graviola, **MSG**: monosodium glutamate**.**

Table (3): Outcome on serum enzymes**.**

Parameters	C	G	MSG	G+MSG	MSG+G
ALT(U/L)	30.71^{a}±2.20	29.19^{a}±1.02	59.54^{b}±3.42	44.11^{c}±3.40	45.36^{c}±3.42
AST(U/L)	27.46^{a}±1.90	29.55^{a}±1.11	66.26^{b}±3.27	43.62^{c}±3.42	47.41^{c}±3.42
ALP(U/L)	131.6^{a}±2.50	130.0^{a}±2.54	169.8^{b}±2.53	150.5^{c}±2.50	149.0^{c}±2.55
GGT(U/L)	20.18^{a}±0.50	20.05^{a}±0.54	28.51^{b}±0.53	25.00^{c}±0.50	25.41^{c}±0.55
TB(mg/dl)	0.45^{a}±0.05	0.40^{a}±0.05	1.27^{b}±0.10	0.76^{c}±0.04	0.77^{c}±0.07
TP(g/dl)	12.59^{a}±0.24	12.79^{a}±0.25	8.42^{b}±0.21	10.30^{c}±0.24	10.00^{c}±0.27
Alb(g/dl)	3.80^{a}±0.14	3.85^{a}±0.15	2.00^{b}±0.11	3.20^{c}±0.14	3.00^{c}±0.17

Results are presented as means ± SE for 6 rats in each group, similar letters (non-significant), Different letters (significant), change at P≤0.05. **C**: control group, **G**: Graviola, **MSG**: Monosodium glutamate.

Table (4): Outcome on hepatic tissue enzymes**.**

Parameters	C	G	MSG	G+MSG	MSG+G
ALT(U/g)	59.46^{a}±3.09	57.40^{a}±3.06	88.46^{b}±3.05	72.79^{c}±3.09	74.99^{c}±3.09
AST(U/g)	$50.36^{a\pm}$ **2.71**	48.21^{a}±2.55	89.70^{b}±3.09	66.76^{c}±2.29	70.52^{c}±3.09
ALP(U/g)	19.81^{a}±1.37	18.95^{a}±1.30	39.41^{b}±1.33	26.22^{c}±1.37	26.46^{c}±1.37
GGT(U/g)	18.43^{a} ±0.52	18.26^{a}±0.52	33.92^{b}±1.16	25.15^{c}±0.52	25.02^{c}±0.71

Results are presented as means ± SE for 6 rats in each group, similar letters (non-significant), Different letters (significant), change at P≤0.05. **C**: control group, **G**: Graviola, **MSG**: Monosodium glutamate.

Table (5): Outcome on hematological parameters

Parameters	C	G	MSG	G+MSG	MSG+G
RBCs(10^6/µL)	9.22^{a}±0.56	9.27^{a}±0.49	4.06^{b}±0.26	6.00^{c}±0.30	5.93^{c}±0.46
Hb (g/dl)	15.46^{a}±0.31	15.92^{a}±0.35	8.50^{b}±0.32	11.56^{c}±0.33	10.54^{c}±0.34
Hct%	51.02^{a}±1.04	52.54^{a}±1.05	28.05^{b}±1.08	38.15^{c}±1.04	34.78^{c}±1.06
MCH (pg)	7.28^{a}±0.86	6.97^{a}±0.59	17.50^{b}±1.28	7.22^{c}±1.06	7.47^{c}±1.05
MCV	46.18^{a}±1.16	45.33^{a}±1.33	59.03^{b}±1.50	47.42^{c}±1.04	48.91^{c}±1.02
WBCs(10^3/µL)	9.50^{a}±0.31	10.20^{a}±0.33	5.45^{b}±0.32	7.25^{c}±0.31	7.07^{c}±0.34
Lymphocyte%	73.12^{a}±1.155	74.20^{a}±1.102	64.92^{b}±1.197	67.00^{b}±1.789	68.00^{c}±1.789
Neutrophil%	18.0^{a}±1.3	18.1^{a}±1.9	7.0^{b}±1.1	16.4^{c}±1.8	16.9^{c}±1.1
PLTs(10^3/µL)	755.9^{a}±35.38	764.3^{a}±40.90	269.6^{b}±25.23	458.4^{c}±46.40	443.3^{c}±44.20

Discussion:

The present inquiry found that for four weeks oral administration of MSG (100 mg / kg bw) resulted in significant disruption in all studied hepatotoxicity parameters studied. On the other hand, oral administration of graviola (200 mg / kg bw) resulted in marked improvement. MSG has caused a significant increase in body weight and this increase can be due to the fact that MSG can boost the palatability of foods by having a positive impact on the center of appetite [15]. MSG's main effect on body weight is by increasing the expression in visceral adipose tissue of interleukin-6 micro-RNA (mRNA), factor-alpha, resistin, and tumor necrosis. Therefore, the increased serum resistin and insulin levels in the visceral adipose tissue can deteriorate glucose tolerance [15]. This in turn results in increased levels of insulin and resistin in the bloodstream and eventually a decreased tolerance to glucose [16]. In fact, the absorption of MSG has a regional effect; when it is detected in the gastrointestinal tract, it stimulates both the celiac and the gastrointestinal branches of the vagus nerve, which activates the limbic system, hypothalamus, insular cortex and nucleus tracts. **[15].** In addition, the consumption of MSG induces a disturbed energy balance by raising the palatability of food and disrupting the cascade of leptin-mediated hypothalamus signaling, potentially resulting in obesity [16]. Glutamate is usually an amino acid that activates the taste receptors of the digestive tract [17, 18]. Therefore, MSG causes thermogenesis in rat brown adipose tissue that is caused by diet and organizes the release of certain hormones such as norepinephrine [16]. MSG stimulates glucagon-like peptide-1 in humans [17] as well. While glutamate decreases the deposition of white-fat in adult rats [18] and can pigs [19]. Thus, MSG increases weight by increasing the intake of food by enhancing the chemosensor. In this research oral administration of graviola (G) (For four weeks, 200 mg / kg bw) returned body weight close to normal level as no significant change was observed when compared with the control group. This finding is in line with the findings of [16-18]. G's decrease in body weight can be due to a decrease in lipid profile such as TG and TC [16,17] whose graviola has a hypolipidimic effect and has hypolipidemic agents such as tannins leading to lower cholesterol absorption [18], leading to a decrease in body weight gain.

There was a significant increase in lipid profile parameters including TC, TG and LDL-C in the serum of rats administered MSG as compared to reduced HDL-C concentration in lipid profile. Such findings are consistent with[16-18]. This was due to the ability of MSG to increase 3-hydroxyl-3-methylglutaryl coenzyme A (HMG CoA) reductase activity., What is the enzyme limiting level in cholesterol biosynthesis leading to increased synthesis of cholesterol. It was stated that hyperlipidemia can transfer the glucose metabolism to lipogenesis with increased serum TG and TC [16]. By addition, an earlier study showed that MSG can alter the expression of adiposity, homeostasis of insulin, hepatic and adipose gene tissue in mice trans-fatty acid [17].

The results of this study also showed that the increased lipid profile parameters caused by MSG were enhanced by oral administration of GE as (TG, TC and LDL-C also increased the concentration level of HDL-C) in the serum. These results are consistent with [17-19], They clarified that hypolipidimic agents present in the GE may be allocated a decrease in TC and TG levels. **[17].** In addition, the presence of certain chemical compounds in the crop, such as coumarins, flavonoids and triterpenoids, may be responsible for changes in biochemical parameters in the hepatic tissues **[18]**. G may have microsomal acetyl coenzyme A inhibitory activity resulting from β-oxidation of fatty acid [19]. The authers [20] confirmed that G has a hypolipidimic effect due to the presence of antioxidants and hypolipidemic agents such as tannins and other polyphenolic compounds coumarins, triterpenoid saponins, flavonoids and other secondary metabolites.

In this study, the serum and liver enzymes ALT, AST ALP and GGT was significantly increased and the animal community treated with MSG were significantly reduced throughout TP and Alb material. Such findings are consistent with [19-23]. The increased levels of ALT, AST ALP and GGT enzymes may be due to the cytotoxic effect of MSG

resulting in damage in the liver cytoplasm and canacules and the release of these enzymes in to blood stream. The elevation because ALT and AST are found in the hepatocyte cytoplasm **[19]**. MSG dissociates into Na ion and glutamate, this glutamate produces ammonium ions that cause toxicity to the liver resulting in damage to the liver tissue, thus increasing the levels of serum liver enzymes. **[20]**. Overloading of the ammonium ion also induces the formation of ROS, which react with polyunsaturated fatty acids found in cell membranes leading to deterioration of plasma and mitochondria membranes accompanied by the release of liver enzymes [21]. Therefore, the rise in serum enzymes could have arisen from the liver damage and oxidative stress caused by MSG [20]. So many cytosolic enzymes in the liver leaked into the blood [22]. Moreover, the rise in ALP and GGT may be due to increased biliary stress and increased production [23]. While erythrocyte hemolysis may be due to the elevation in TB levels [24]. The decrease in TP and Alb showed the degenerative impact of MSG on hepatic cells [25].

On the other hand, oral GE administration minimized the destructive effect of MSG at liver function as GE decreased ALT, AST, ALP, GGT enzymes as well as TB and increased TP and Alb content. This can be attributed to the capacity of the extract to maintain the structural integrity of the hepatocytic cell membrane or to regenerate the damaged liver cells [26].

In this study, the administration of MSG resulted in a significant decrease in Hb concentration, RBCs, Hct level, platelets and WBCs, lymphocytes and neutrophils, a significant increase in MCV and MCH compared to control group. Such data are consistent with the [23-25] results.. The decline in Hb, RBCs, and WBCs may be due to MSG resulting in a drop in RBCs ' half-life duration, which may be due to the direct toxicity of MSG salt on RBCs contributing to its hemolysis. It may also be due to the salt effect of MSG in the bone marrow (the main production site) on RBC stem cells. In addition, degradation of RBCs may also be proposed as a result of increased oxidative stress in tissues caused by MSG water. Consequently, reducing the number of RBCs can result in a significant decrease in Hb concentration [23]. The decrease in WBCs may be due to the reduction of MSG mediated immunological function [24]. And, the spleen and thymus damaging influence of MSG. the authers recorded that increasing glutamate can harm lymphocyte function and stimulate secondary immunopathological effects which lead to mitogenin deactivation which induces lymphocyte proliferation [23,24]. The drop in lymphocytes and neutrophils in groups treated with MSG is an indicator of compromised immune function and toxicity compared to control [23]. Furthermore, the increase in MCV showed that the cells are macrocytic which are a marker for megaloblastic anemia and pernicious anemia [24] while increase in MCH is an indicator for macrocytic anemia (normochromic) [25]. Therefore, pernicious anemia has been observed (normochromic macrocytic anemia) and this may be due to the atrophy induced by MSG in gastric mucosa (gastritis) as the L-form of glutamic acid is acidic, resulting in a decrease in the synthesis of the intrinsic factor, resulting in vitamin B12 malabsorption and this is the main cause of pernicious anemia [23]. The increased value of neutrophils with MSG also showed toxicity and inflammation induced by MSG. On the other hand, our study showed that oral G administration led to a significant increase in Hb concentration, RBCs, WBCs, lymphocytes, neutrophils compared to groups treated with MSG. This finding is consistent with the data obtained by [23, 24] who clarified that graviola was capable of treating diarrhea, stopping rats from losing body fluid. G also has the ability to induce the release of erythropoietin, the regulatory hormone in RBC development from the kidney [23]. Increasing WBCs and platelets may also be triggered by the GE's hepatoprotective effect. GE administration has also enhanced the percentage of Hct and these findings are close to data obtained by [23, 24] due to increased concentration of Hb.

4. References

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