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Effect of *Myrtus Communis* Extract against Hepatotoxicity Induced by Monosodium Glutamate and Acrylamide in Male Rats

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ABSTRACT

Background: Myrtle leaves extract (ME) has many antioxidants that protect against oxidative stress. The current study was conducted to determine whether ME can possibly exert hepatoprotective and antioxidant activity against monosodium glutamate (MSG) and acrylamide (ACR) that induced toxicity in rats.

Objectives: Our experiment was carried out to examine the effect of *Myrtus communis extract* against hepatotoxicity stimulated by monosodium glutamate (MSG) and acrylamide (ACR) in male rats.

Materials and methods: Rats were randomly assigned into eight groups, containing six each as following: group 1: rats received dist. water (control); group 2: rats were orally administered myrtle extract (ME) (300mg/kg b w) daily for 7 wks.; groups 3, 4 and 5: rats were orally administered MSG (100mg/kg b w), ACR (20mg/kg b w) and (MSG + ACR) respectively daily for 6 wks. ; groups 6 ,7 and 8: rats were orally administered ME daily for seven days alone then associated with MSG or with ACR or with (MSG+ACR) respectively for 6 wks.

Results: Our results proved that the treatment with MSG and/ or ACR resulted in a significant rise in TL, TC, TG, LDL-C, ALT, AST, ALP, GGT, TB and MDA. However, marked reduction in HDL-C, TP, Alb, GSH, TAC, SOD, CAT and GSH-Px. On the other side, the administration of ME improved the deviations resulted from MSG and/or ACR as confirmed by the marked improvement of antioxidants.

Conclusion: It is concluded that ME could protect the liver against damage induced by MSG and ACR.

Keywords: *Myrtus communis* , Monosodium glutamate, Acrylamide, Hepatotoxicity

Abbreviations: ME ,myrtle extract; MSG, monosodium glutamate; ACR, acrylamide; TL, total lipids; TC ,total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TP, total protein; Alb, albumin; TB, total bilirubin; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase.; MDA, malondialdehyde; GSH, reduced glutathione; TAC, total antioxidant capacity, SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase.

INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of glutamic acid , glutamic acid(78%) sodium and water(22%). MSG is generally used as food additive or a flavor enhancer. Chronic administration of MSG can lead to oxidative stress, brain disorders, renal toxicity and hepatic damage[1].

Acrylamide (ACR) is an α–β unsaturated carbonyl compound**.** It is used in grouts, packing, water purification, paper manufactures, soil stabilizers and cosmetic industries. Furthermore, ACR is formed in foodstuff containing the amino acid asparagine and reducing sugars**.** ACR is dangerous pollutant due to its neurotoxic, carcinogenic, hepatotoxic and may cause mutation in male germ cells[2].

Myrtus communis, commonly known as myrtle, is an evergreen plant commonly scattered in Europe, America, Africa and Asia. Myrtle is found to be rich in polyphenolic compounds such as tannins and flavonoids. Several extracts of myrtle have been found to have anti-hyperglycemic ,anti-inflammatory and antioxidant activities in different studies[3].

MATERIALS AND METHODS

Chemicals

Monosodium glutamate was obtained from Metro market, Mansoura, Egypt. MSG was dissolved in dist. H_2O and orally administered (100mg/kg b w). The chosen dose of MSG was according to the previous studies[4]**.** Acrylamide was purchased from Sigma Chemical Company (St. Louis, MO, USA). ACR was dissolved in dist. H_2O and orally administered (20mg/kg b w). The chosen dose was according to the previous studies[5]**.**

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Myrtle extract

Leaves of myrtle were collected from the Orman garden, Giza, Egypt. The leaves were washed with water several times and dried in shed at 25C° for two weeks with continuous turning over. Dried leaves were powdered mechanically and stored in airtight containers. About 250 g of the powdered leaves were soaked in 1000 ml of H_2O for seven days with continuous stirring. The mixture was filtrated with filter paper Whatman No 1.The filtrate was concentrated at reduced pressure vacuum using Rotary Evaporator. The extract weighed and dissolved in dist. H_2O to prepare (300 mg extract /kg b w). The selected dose of ME was according to the previous study[6].

Animals

White male albino rats (*Rattus norvegicus*) 7-8weeks old, with average weight 160-170g. They were obtained from the animal house of National Research Institute, El-Giza, Egypt. The rats were housed in stainless steel cages under standard hygienic conditions with good ventilation at (24°C). The rats were served with water *ad libitum* and rat chow and were acclimatized prior to experiment for 7 days.

Animal grouping

Forty eight rats assigned into eight groups (containing six each) and orally administered the treatment by using the stomach tube for 7 wks. as following: group 1: control rats group received dist. H_2O; group 2: rats were administered ME (300mg/kg b w) daily for 7 wks.; group 3, 4 and 5: rats were administered MSG (100mg/kg b w), ACR (20mg/kg b w) and MSG + ACR respectively daily for 6 wks. ; groups 6, 7 and 8: rats were administered ME for seven days then associated with MSG, ACR and MSG+ACR respectively for 6 weeks.

Sample collection

Twenty four hr. after last treatment. Rats were sacrificed and blood samples were collected in clean centrifuge glass tubes, left to clot then centrifuged at 860 rpm for 15 min. The clean non hemolysed supernatant was quickly removed .The sera were kept in deep freezer at -20^{o}C for numerous parameters. Rat liver was removed, weighed for each rat. Liver tissues were homogenized (10% w/v) in dist.H_2O. The samples were labelled and kept at -20^{o}C till used.

Investigated parameters

These parameters were estimated in the serum and hepatic tissues: TL[7], TC[8],TG [9], TP[10], ALT and AST[11], (ALP)[12], GGT[13]. While HDL-C[14], LDL-C[15], Alb[16], TB[17] were estimated in the serum only .Wherever ,the following parameters were estimated in the hepatic tissues: MDA[18], GSH[19], TAC[20] , SOD[21], CAT[22], GSH-PX[23].

Statistical analysis

All the statistical analyses were performed by SPSS 15.00 software. Data gotten from the research were studied by means of one- way analysis of variance (ANOVA), followed by Duncan′s tests[24]. Differences were considered significant at $P \leq 0.05$.

Ethics

Animal procedures as handling, housing and prosperity of the rats used for the study were in agreement with the Ethics and Regulations guiding the use of animals for research in the University of Mansoura. All efforts were made to minimize animal suffering.

The study was approved by the Ethics Board of Mansoura University.

RESULTS

oral treatment with MSG and/ or ACR caused marked rise in serum TC, TG, TL, LDL-C, and TB, in addition to hepatic TC, TG, TL and reduction in HDL-C, TP and Alb. as presented in Table (1). As well as, data indicated in Table (2). There was a significant rise in (ALT, AST, GGT and ALP) activities in both serum and hepatic tissue in MSG and/ or ACR rats.

But, in Table (3) data were documented marked elevation in MDA content and notable decline in GSH, TAC, SOD, CAT and GSH-Px in MSG and/or ACR rats. Whereas, the treatment with ME before and concomitant with MSG and/ or ACR improved the hepatic oxidative stress markers and biochemical variations as well as improvement of the antioxidants (Tables 1–3).

Table 1
Effect of ME on serum and hepatic biochemical parameters in experimental rats.

Parameters	C	ME	MSG	ACR	MSG+ACR	ME+MSG	ME+ACR	E+MSG+ACR
Serum								
TL (mg/dl)	33.67±3.18[a]	33.52±2.51[a]	25.68±6.17[b]	79.15±8.03[c]	83.16±10.59[d]	54.49±5.76[a]	34.60±7.43[e]	630.05±7.38[f]
TC (mg/dl)	01.58±0.78[a]	01.09±0.85[a]	34.00±1.24[b]	42.17±1.35[c]	58.85±2.81[d]	06.17±2.74[a]	09.83±2.44[e]	133.00±1.76[f]
TG (mg/dl)	8.50±2.49[a]	8.33±2.51[a]	28.34±1.25[b]	39.17±1.14[c]	48.83±1.70[c]	8.37±2.50[a]	20.24±8.44[d]	136.02±1.85[e]
HDLC (mg/dl)	1.42±0.90[a]	2.58±0.74[a]	4.09±1.04[b]	2.44±1.43[c]	21.09±1.12[c]	0.75±1.14[a]	1.92±0.58[d]	36.50±0.76[b]
LDL-C (mg/dl)	4.53±0.44[a]	4.45±0.49[a]	6.08±1.21[b]	03.85±2.27[c]	21.11±5.02[d]	9.44±1.03[a]	0.96±1.41[e]	50.96±1.41[e]
TP (g/dl)	0.22±0.23[a]	0.25±0.26[a]	7.26±0.12[b]	6.43±0.07[c]	5.53±0.11[d]	0.56±0.21[a]	9.47±0.07[e]	8.21±0.24[f]
albumin (g/dl)	3.85±0.05[a]	3.86±0.08[a]	3.23±0.05[b]	3.16±0.01[b]	2.63±0.04[c]	3.82±0.04[a]	3.49±0.03[d]	3.33±0.04[b]
bilirubin (mg/dl)	0.55±0.01[a]	0.54±0.01[a]	1.01±0.05[b]	1.73±0.09c	2.39±0.11[d]	0.54±0.01[a]	0.55±0.01[a]	0.67±0.01[a]
Liver								
TL (mg/g)	28.24±7.67[a]	28.01±7.07[a]	72.86±9.03[b]	74.81±7.45[c]	07.98±29.75[d]	35.67±8.11[a]	78.28±9.56[e]	2035.33±7.42[f]
TC (mg/g)	15.00±0.97[a]	14.39±0.82[a]	28.50±1.18[b]	30.67±1.05[c]	36.83±1.45[d]	17.00±1.64[a]	19.73±0.80[e]	126.33±1.15[f]
TG (mg/g)	5.67±16.26[a]	12.50±2.07[a]	07.17±3.64[b]	10.00±2.89[b]	63.33±8.82[c]	34.17±1.65[a]	64.33±1.45[d]	389.83±5.39[e]
TP (g/g)	5.80±0.05[a]	5.82±0.10[a]	4.10±0.05[b]	3.66±0.09[c]	3.15±0.08[c]	5.65±0.07[a]	4.68±0.11[d]	4.33±0.08[e]

Data were expressed as means ± SE (number of rats in each group 6).
Different Superscript letters (a-f) indicated the significant change at p≤0.05 (Duncan′s Test)
C: control ME: myrtle extract MSG: monosodium glutamate ACR: acrylamide

Table 2 : Effect of ME on serum and hepatic enzymes activities in experimental rats .

Parameters	C	ME	MSG	ACR	MSG+ACR	ME+MSG	ME+ACR	ME+MSG+ACR
Serum								
ALT (U/L)	**24.93±0.58[a]**	**24.01±0.39[a]**	**50.88±0.77[b]**	**54.62±0.44[c]**	**62.28±0.71[d]**	**27.63±0.68[a]**	**32.73±0.74[e]**	**46.03±0.73[f]**
AST (U/L)	**34.20±1.07[a]**	**33.03±0.84[a]**	**50.83±0.74[b]**	**59.55±0.69[c]**	**68.03±1.08[d]**	**34.17±1.11[a]**	**34.67±1.03[a]**	**41.37±0.45[e]**
ALP (U/L)	**119.83±0.95[a]**	**119.00±1.06[a]**	**166.33±2.17[b]**	**175.67±1.65[c]**	**184.33±1.54[d]**	**120.42±0.61[a]**	**129.23±0.86[e]**	**133.50±1.46[f]**
GGT (U/L)	**23.05±0.53[a]**	**23.00±0.50[a]**	**36.67±2.25[b]**	**44.00±1.86[c]**	**48.33±1.94[d]**	**22.87±0.39[a]**	**23.41±0.33[a]**	**25.98±0.32[a]**
liver								
ALT (U/g)	**51.15±0.38[a]**	**51.00±0.47[a]**	**63.05±0.58[b]**	**82.75±0.54[c]**	**87.42±0.62[d]**	**53.96±0.29[a]**	**54.90±0.50[a]**	**65.93±0.39[e]**
AST (U/g)	**61.92±0.60[a]**	**61.00±0.51[a]**	**82.26±0.56[b]**	**86.13±0.68[c]**	**92.73±0.72[d]**	**62.47±0.45[a]**	**62.00±0.62[a]**	**65.87±0.38[e]**
ALP (U/g)	**15.24±0.58[a]**	**14.77±0.56[a]**	**32.42±0.62[b]**	**43.08±0.89[c]**	**54.63±1.20[d]**	**15.25±0.59[a]**	**18.67±0.42[e]**	**23.33±0.59[f]**
GGT (U/g)	**24.39±0.20[a]**	**24.34±0.52[a]**	**29.02±0.59[b,e]**	**36.67±1.76[c]**	**46.17±1.25[d]**	**24.14±0.55[a]**	**25.86±0.48[a,e]**	**27.06±0.79[e]**

Data were expressed as means ± SE (number of rats in each group 6).
Different Superscript letters (a-f) indicated the significant change at p≤0.05 (Duncan′s Test)
C: control ME: myrtle extract MSG: monosodium glutamate ACR: acrylamide

Table 3: Effect of ME on antioxidant biomarkers in experimental rats.

Parameters	C	ME	MSG	ACR	MSG+ACR	ME+MSG	ME+ACR	ME+MSG+ACR
Liver								
MDA (nmol/g)	**838.28±14.84^{a}**	**836.71±15.97^{a}**	**1140.81±14.86^{b}**	**1187.50±3.82^{c}**	**1210.00±5.32^{c}**	**861.19±10.31^{a}**	**914.54±14.54^{d}**	**956.84±16.00^{e}**
GSH (mmol/g)	**4.53±0.15^{a}**	**4.55±0.18^{a}**	**3.65±0.08^{b}**	**2.47±0.13^{c}**	**1.66±0.07^{d}**	**4.23±0.19^{a}**	**3.39±0.21^{e}**	**2.24±0.04^{c}**
TAC(mM/g)	**1.45±0.01^{a}**	**1.46±0.02^{a}**	**1.36±0.01^{b}**	**1.24±0.01^{c}**	**1.16±0.01^{d}**	**1.36±0.01^{b}**	**1.32±0.02^{b,d}**	**1.24±0.01^{c}**
SOD (U/g)	**173.83±1.82^{a}**	**173.90±1.86^{a}**	**158.17±2.18^{b}**	**145.76±1.58^{c}**	**116.50±2.13^{d}**	**168.67±2.78^{e}**	**166.90±3.35^{e}**	**139.17±3.00^{c}**
CAT(U/g)	**195.00±1.63^{a}**	**196.67±0.88^{a}**	**134.17±1.01^{b}**	**125.33±2.91^{c}**	**117.83±1.01^{d}**	**191.67±2.60^{a}**	**182.00±1.98^{e}**	**162.50±2.05^{f}**
GSH-Px(U/g)	**756.29±6.37^{a}**	**757.53±7.22^{a}**	**644.34±6.80^{b}**	**622.70±3.77^{c}**	**592.00±6.51^{d}**	**740.66±10.25^{a}**	**685.25±3.51^{d}**	**647.50±6.29^{b}**

Data were expressed as means ± SE (number of rats in each group 6).
Different Superscript letters (a-f) indicated the significant change at $p \leq 0.05$ (Duncan′s Test)
C: control ME: myrtle extract MSG: monosodium glutamate ACR: acrylamide

DISCUSSION

The present investigations showed that the oral administration of MSG and/or ACR for 6 wks. led to marked disturbances in all the tested parameters indicating induction of hepatotoxicity. On the other hand, administrations of ME extract before and concomitant with MSG and/or ACR caused marked amelioration. The negative effects of MSG in the liver could be attributed to generation of ROS that resulted in oxidative stress and impaired antioxidant system. MSG increased electron flow obstruction and enhanced degenerative diseases by generation of free radicals**.**Moreover, MSG dissociated to yield free glutamate. By oxidative deamination of glutamate produces ammonium ion $(NH_4)^+$ that could be toxic unless detoxified in the liver via the reactions of the urea cycle. So, the ammonium ion excess after MSG consumption could damage hepatic cells[25]**.** Alternatively, ACR is metabolized to glicidamide (GA) by P450, binds to DNA, it is more reactive toward proteins and DNA than ACR and it leads to genotoxicity**.** Additionally, forming of glicidamide-DNA adducts could be participated in acrylamide-induced, mutagenicity, toxicity and carcinogenicity. ACR is easily absorbed and metabolized in the body and excreted as metabolites in the urine**.** It is able to interact with vital cellular nucleophiles having OH, SH, or NH_2. So, ACR transformation happens through GSH conjugation and decarboxylation[26]**.**

On the other hand, ME extract has phenolic compounds such as carvacrol, rosmarinic acid and thymol cause a threefold increase in O-methylguanine-DNA methyltransferase (MGMT)**.** MGMT is the first defense mechanism against DNA damage and physically it deals with cell proliferation, nucleus antigen and nucleus polymerase during the DNA replication [27]**.**

The observed dyslipidemia in our experiment because of treatment with MSG evident by increase of TC, TG, TL, LDL-C and a decrease of HDL-C. .MSG was seen to increase hepatic lipid catabolism via up regulation of oxidative genes especially those involved in bile acid pathway including key regulatory enzyme, cholesterol-7-α hydroxylase (CYP7A1)[28]**.**

Meanwhile, the observed hyperlipidemia from ACR administration may be due to an increase in the synthesis of plasma lipoproteins and high mobilization of lipids from the liver. Fried food contains high concentration of trans-fat that can increase the concentration of LDL-C [29]**.**

On the other hand, administration of ME before and concomitant with MSG and/or ACR improved the lipid profile, ME has strong hypolipidemic activity, which may be due to the presence of important substances as linoleic acid, oleic acid and stearic acid**.** In addition, the presence of semi myrtucommulone and myrtucommulone in ME may have a role in hypolipidemic and decrease oxidative stress activity[30]**.**

In our study, the daily intake of MSG and/or ACR resulted in a significant increase in serum and hepatic ALT, AST, GGT and ALP activity which might be due to the damage of hepatocytes. Numerous of hepatic enzymes exist in the cytosol leaked into the blood when the hepatocyte membrane is damaged**.** Also, the elevated activities of these enzymes in ACR intoxicated rats could be attributed to the bipolar nature

of ACR, where the CH_2=CH part may undergo hydrophobic interactions while the $CONH_2$ part and form hydrogen bonds with the cell compounds. This property of ACR may alter the cell membrane structure and make the parenchymal cell membrane of hepatocytes more permeable causing the active retention of enzymes and making them appear first in the extracellular space and then in the blood[31].

Furthermore, the increased ALP activity might be due to increasing biliary pressure and elevated production[32]. Daily intake of MSG and ACR showed an increase in serum TB and a decrease in Alb and TP when compared to control rats. Alb and TP are indicators of biosynthesis function of the liver and the decrease of these proteins reflect the damage of liver. While the increased levels of TB may be attributed to an increased production, possible erythrocyte hemolysis or decreased uptake by the liver[33] .

On other hand, administration of ME improved liver function probably due to the flavonoids present in myrtle leaves such as rosmarinic acid which are able to neutralize free radicals and prevent its damaging effects by their antioxidant property. Moreover, carvacrol has increased regeneration rate of the hepatic tissue[34].

The present investigation revealed that oral administration of MSG and/or ACR suppressed SOD, CAT, GSH-Px activities and GSH content while, there was an elevation in LPO level in the liver tissues. Increase of LPO because of the elevation of the blood glutamate and glutamine which favor lipogenesis. Glutamine degradation produces glutamate which then subjected to oxidative deamination to yield ammonium ions in the liver. Subsequently, by changing the redox potential of the cell the glutamine initiates LPO. The increase MDA level here in, is indicator for the oxidative stress exerted in the liver[35].

Moreover, the decreased content of GSH in rats which administrated MSG and/or ACR may be attributed to the increased LPO. Also, the observed decreased content of GSH due to the most hepatic GSH is converted to GSSG by glutathione reductase to keep the cells from injury by the free radicals and toxic materials. While, SOD activity depletion was attributed to its inhibition by accumulation of H_2O_2. So, reduction of SOD activity and the rise of MDA level could be signs of oxidative stress[36].

Otherwise, oral administration of ME before and concomitant with MSG and/or ACR have improved the antioxidant status may be through increasing the antioxidants; GSH, SOD, CAT, GSH-Px and decreasing the oxidative stress. This may be due to stimulation of DNA polymerase by the flavonoid compounds in myrtle leaves extract in addition to increasing rRNA synthesis and repair of liver cells[37]. Also, timolol and carvacrol, two important compounds of myrtle leaf extract that increase glucose-6-phosphate dehydrogenase (G-6 phD) enzyme which is the main regulatory enzyme of pentose phosphate pathway. This pathway provides NADPH necessary for the decomposition of H_2O_2. H_2O_2 is removed by GSH-Px which acquires its required hydrogen by oxidizing glutathione. The reduction of glutathione is performed by glutathione reductase which receives the required energy from phosphogluconate pathway. With increased activity of the pentose phosphate pathway. Elimination of H_2O_2 increases where this response is accompanied by the increase of SOD, CAT and GSH-Px[38].

CONCLUSION

The present results highly submitted the effectiveness of ME supplementation as useful hepatoprotective agent against disturbance of liver and the oxidative stress caused by MSG and ACR. This may be due to their antioxidant and scavenging properties towards free radicals.

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