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### RESEARCH ARTICLE

## AMELIORATING EFFECTS OF MARJORAM LEAVES EXTRACT AGAINST HEPATIC DISORDER IN MALE RATS.

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### Abstract

The present study aims to evaluate the protective role of marjoram (M) leaves extract against hepatic disorder- induced by thioacetamide (TAA) in male rats. Male Wister rats (150-200 gm) were divided into eight groups (6 rats / each). Group 1 normal control. Groups 2 and 3 received 200mg and 400 mg/kg bw ethanolic extract of marjoram (M) leaves respectively. Group 4 received 100mg/kg of silymarin (Sly). Group 5 received 200mg/kg/ bw of TAA. Group 6 received M200 with TAA. Group 7 received M400 with TAA. Group 8 received Sly with TAA. All treatments continued for 8 weeks. The results showed that treatment with TAA caused decrease in the body weight and increase liver weight. Also, the results showed changes in the hepatic function biomarkers which characterized by increased activities of serum enzymes (ALT, AST, ALP, GGT) and total bilirubin associated with decrease in total protein and albumin contents. Also, increased serum levels of total lipid (TL), total cholesterol (TC), triglycerides (TG) and LDL-C with significant decrease in HDL-C. Moreover, the results showed significant decrease in hepatic TAC, SOD, GSH, GST and CAT activities while hepatic MDA level showed significant increase in TAA group. Treatment of animals with ethanolic extract of M in doses of 200 mg and 400 mg/ kg bw significantly reduced the TAA induced changes in the serum enzymes activities, lipid profile and antioxidants to near normal values. In conclusion, the results of the study indicated that the ethanolic extract of M has hepatoprotective activity against TAA induced hepatotoxicity in rats compared to Sly. The hepatoprotective effect of M may be due to the different antioxidant constituents that reduce oxidative stress and in hence the antioxidant status.

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### Introduction:-

The liver is the largest organ in the body, it is the premier chemical factory necessary for survival. It is the first stop for all nutrients, toxins and drugs absorbed by digestive tract. The liver also plays vital role in metabolism and has a lot of functions in the body (1). Liver diseases are among more serious ailments affecting a large population of world and can be classified as acute or chronic hepatitis and cirrhosis. Hepatotoxicity implies chemical-driven liver damage and can occur due to drug overdose and sometimes even in therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries can also induce hepatotoxicity (2).

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TAA is a thiono-sulfur containing compound. It has been used as a fungicide, organic solvent, accelerator in the vulcanization of rubber, and as a stabilizer of motor oil (3). Physiologically, it is known that TAA toxicity is generally associated with hepatic fibrosis induction, complicated metabolic disorders and health problems (4).

TAA has long been known as a hepatotoxicant; its biotransformation to thioacetamide sulfoxide (TAAS) occurs along the cytochrome P-450

(CYP)-dependent pathway; TAAS is subsequently converted to thioacetamide disulfoxide, a toxic reactive metabolite. This reactive metabolite covalently bind to liver macromolecules and dramatically increases the production of reactive oxygen species (ROS) which then induce acute centrilobular liver necrosis (5).

From ancient times the medicinal properties of plants were discovered. Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants, (6). The hepatoprotective effects are associated with phytochemicals rich in natural antioxidant (7). Several bioactive constituents from medicinal plants have been investigated for hepatoprotective and antioxidant effects against hepatotoxin-induced hepatic disorder (8). Natural medicinal plants are seen as safe and effective alternative therapies for liver damage (9).

*Origanum majoranum* L. (Marjoram) is one the most common member of the mint family Lamiaceae and of the most popular culinary herbs in the world, which was grown in Egypt over 3,000 years ago where 90% of the world's supply is produced by Egypt (10). Marjoram is used as a home remedy for chest infection, cough, sore throat, rheumatic pain, nervous disorders, stomach disorders, cardiovascular diseases, and skin care (11). Several studies have shown that ethanolic, aqueous extracts and essential oil of *Origanum majorana* could protect against liver and kidney damage and genotoxicity induced by lead acetate (12).

Silymarin a polyphenolic flavonoid isolated from "milk thistle" has clinical applications in the toxic hepatitis treatment, fatty liver, cirrhosis, ischaemic injury, radiation toxicity and viral hepatitis as a result of its antioxidative, anti-lipid-peroxidative, antifibrotic, anti-inflammatory, immuno-modulating and even liver regenerating effects (13). In experiments on animals, Silymarin and Silibinin are shown to protect rat and mouse liver against hepatotoxicity induced by acute thioacetamide (14).

Accordingly, the present study aims to investigate the possible hepato-protective effects of ethanol extract of marjoram against TAA-induced hepatotoxicity in comparison to silymarin as a standard drug.

## Materials and Methods:-

### Chemicals:-

TAA was purchased from (sigma Aldrich, USA) chemicals. Sly was purchased from a pharmacy as tablets from MADAUS pharmaceuticals Cairo, Egypt. All used other chemicals were of analytical grade.

### Preparation of Marjoram (M) extract:-

M dry leaves were purchased from Alhraz market, Cairo, Egypt, powdered, grinded then stored in airtight- sterile container and kept in room temperature. Marjoram ethanolic extract was prepared from M powder according to (15), 500gm of M powder was mixed with 1500 ml of 95% ethanol, stirred and repeated twice. The extract was filtered with Whatman No 1 filter paper then evaporated to dryness using a rotary evaporator under vacuum. The residue was stored at 4 °C until used. A fresh solution of the extract was prepared in distilled water before administration.

### Experimental animals:-

Forty eight Wister male rats, weighting 150-180g were obtained from Helwan animal Farm, Cairo, Egypt and were housed in separate metal cages. They were kept under normal laboratory circumstances (12h: 12h light /dark cycles at 25±2 °C) with free access to standard pellet food and water *ad libitum*. The animals were acclimatized for 2 weeks before beginning the experimental study.

### Experimental design:-

The experimental rats were divided into 8 groups each of 6 rats as the following:

**Group 1:-**Control group without treatment.

**Group 2:- (M200):** Rats were orally administered M extract (200mg/kg bw) daily.

**Group 3:- (M400):** Rats were orally administered M extract (M400 mg/Kg bw) daily.

**Group 4:-**Rats were orally administered Sly standard drug (100mg/kg bw) daily (16).

**Group 5:-**Rats were IP injected with TAA (200 mg/kg bw) three times a week.

**Group 6:-**Rats were orally administered M extract M200 then injected with TAA with the same doses and route of administration as groups 2 and 5.

**Group 7:-**Rats were orally administered M extract M400 then injected with TAA with the same doses and route of administration as groups 3 and 5.

**Group 8:-**Rats were orally administered Sly then injected with TAA with the same doses and route of administration as groups 4 and 5.

All treatment were continued for 8 wks. Animal body weights were recorded at the start and weekly to adjust dose and to obtain body weight changes.

#### **Blood and liver sampling:-**

At the end of the experimental period, animals fasted overnight then were sacrificed under anesthesia by diethyl ether. Blood samples from each rat were collected into clean centrifuge tubes. After complete blood coagulation, the tubes were centrifuged at 860 xg for 10 min. The separated sera were frozen at -20 °C for future biochemical analysis. The animals were immediately dissected, the liver was removed and weighed to calculate the absolute and relative liver weight. Small, weighted specimens from the liver tissue was homogenized in known volume of phosphate buffer and centrifuged at 860xg for 10 min. Supernatant was obtained and kept frozen at -20 °C for biochemical assay.

#### **Biochemical analysis:-**

Serum ALT and AST activities were determined using RAM diagnostic Kits. Serum ALP, TP, TC, TG, HDL-C, as well as, hepatic SOD, CAT, TAC, GST, GSH and MDA were measured using kits obtained from Biodiagnostic Co. Dokki, Giza, Egypt. While, serum GGT activity was determined using Kit purchased from Vitro Scient Cairo, Egypt. Albumin and total bilirubin were determined using Kit purchased from Diamond Diagnostics Co. Cairo, Egypt. The concentration of serum total lipids was estimated using kit purchased from ABC Diagnostic New Diemetta, Egypt. The LDL-C was calculated according to the equation described by (17).

#### **Statistical analysis:-**

Results were expressed as mean  $\pm$  standard error of mean (SEM). Statistical significance was calculated using one-way analysis of variance (ANOVA) followed by Duncan tests. The values of  $p \leq 0.05$  were considered statistically significant.

#### **Results:-**

In table 1, obtained results, showed that administration of M (200 or 400 mg/kg bw) and Sly each alone caused non-significant decrease in the body weight, absolute and relative liver weights. While administration of TAA caused a significant decrease in body weight associated with increased liver weight comparing with the control rats. However M200 or M400 administration with TAA showed a significant increase in body weight and significant decrease in liver weight comparing to TAA treated group. On the other hand, administration of Sly with TAA showed non-significant changes in the body weight and liver weights reaching them to the control normal levels.

In table 2, TAA treated group showed marked elevation in the serum enzyme activities of ALT, AST, ALP and GGT as well as serum total bilirubin content when compared to control group. Meanwhile, decreased serum total proteins and albumin compared to control group were observed. However, administration of M200, M400 or Sly with TAA showed improvement in the activities of the mentioned enzymes as well as serum total protein, albumin and total bilirubin comparing to TAA group. However, serum total protein and albumin in M400 +TAA and Sly +TAA groups reached to normal levels.

Concerning lipid profile, (table 3) there were a non-significant changes in serum TL, TC, TG, LDL-C and HDL-C concentrations in M extract M200, M400 and Sly groups comparing with the control. Meanwhile, TAA administration caused significant increases in serum TL, TC, TG and LDL-C in contrast to HDL-C which showed significant decrease. However, M200, M400 or Sly with TAA showed a significant reduction in serum TL, TC, TG and LDL-C concentrations comparing to TAA group while serum HDL-C revealed a significant increase.

Table 4, showed non-significant changes in hepatic TAC, SOD, GSH, GST and CAT in M200, M400 and Sly groups comparing to the control group were observed. In contrary, the data revealed a significant increase in hepatic MDA associated with significant decrease in the levels of TAC and GSH, as well as SOD, GST and CAT activities in TAA group comparing to control one.

In case of rats administrated with M200 and M400 with TAA there were significant increases in hepatic TAC, SOD, GSH, GST and CAT while hepatic MDA showed a significant decrease comparing to TAA rat group. However, the hepatic TAC, SOD, GSH, GST and CAT in Sly+ TAA reaching to control normal level. The pervious result indicate that most beneficial effect was observed in M400+ TAA and sly +TAA protected groups.

### Discussion:-

The liver plays an important role in the detoxification of foreign substances, in the secretion of bile for digestion, and in the metabolic functions of various nutrients including carbohydrates, proteins, and fats (18). The liver disorders are a worldwide problem that causes high morbidity and mortality. There is no therapy can successfully control the progression of liver diseases therefore, researches about herbal medicine that could replace the chemical drug were needed (19).

Medicinal plants are promising source of hepatoprotective and antioxidants activity so has been used in the treatment of liver diseases. Marjoram (*Origanum majorana*) (M) is the most important member of the Lamiaceae family, that is used worldwide as a spice and crude drug. It possesses high antioxidant and anticancer properties (20).

Thioacetamide (TAA) is known hepatotoxic, which produces hepatic necrosis in high doses by producing free radicals during TAA metabolism resulting in oxidative stress mediated acute hepatitis and induces apoptosis of hepatocytes in the liver (21).

In the present study, administration of rat with TAA caused a significant decrease in body weight associated with increased absolute and relative liver weights comparing with the control rats. These results are in agreement with (22, 23, and 24) where their studies showed significant decrease in body weight in TAA treated rats accompanied with a notable increase in liver/body ratio. The enlargement of livers in TAA treated rats signified hepatic lesions and liver injury associated with the toxicological effects of TAA. The decrease in mean body weight recorded in TAA treated rats may be adduced to malnutrition resulting from reduced absorption of nutrients from the intestine of treated rats and TAA –induced protein catabolism. These symptoms may be caused by toxic metabolites of TAA (thioacetamide-S-oxide and thioacetamide-S, Sdioxide). The reduction in the body weight and internal organs weights are considered as simple indices of toxicity.

However M (200mg and 400mg) administration with TAA showed a significant increase in body weight and decrease liver weight comparing to TAA treated rat group. Similar observations have been reported by (25) who mentioned that, the oral administration of methanolic extracts of *Origanum majorana* increased body weight and decreased liver weight in CCL<sub>4</sub> treated rats. This improvement may be due to beneficial effect of antioxidant administration against TAA poisoning with respect to body weight observed in the present study confirms previous results obtained by (26) who concluded that feeding rats with antioxidants could play an important role as a prophylactic against the toxic effects of CCL<sub>4</sub>.

In addition, TAA toxicity caused a significant increase in serum enzyme activities (AST, ALT, ALP and GGT) comparing to control group. The current results agreed with (27) who reported that, the administration of TAA for eight weeks induced hepatic disorder due to TAA-intoxication was manifested by marked increase in liver enzyme activities (ALT, AST, ALP, GGT) and total bilirubin content accompanied by a decrease in albumin and total protein content in rats.

Based on the study of (28) who mentioned that, that rats receiving single IP injection of TAA causes increased liver transaminases (AST ALT) and ALP by TAA treatment. Moreover Lebda (29) reported that, after exposure to TAA evidenced increase in serum biomarker (ALT and AST) activities as well as total bilirubin levels associated with significant decrease in total protein, albumin content compared to control rats. These results could expected since TAA-induced necrosis and/ or membrane damage releases these enzymes into circulation.

On other hand, administration of M200, M400 and sly with TAA showed a significant improvement represent by decrease in serum AST, ALT, ALP and GGT activities comparing to TAA treated rats. The effect was pronounced in sly +TAA than M400 +TAA rat. These results in agreement with the findings of (16, 25) they reported that, administration of *Origanum majorana* extract increased the antioxidant enzymes and significantly decreased liver enzyme activities in CCl<sub>4</sub> intoxicated rats. **Baatour (30)** found that marjoram contain several compounds such as phenolic components (thymol, carvacrol) and flavonoids (diosmetin, luteolin, apigenin) that are responsible for this antioxidant effect. Serum levels of these biomarker of liver function are very sensitive markers employed in the diagnosis of liver diseases. Also, (31) reported that, the disturbance of ALP and bilirubin may be due to bile excretion is inhibited by hepatotoxicity which leads to increase of normal values due to body inability to excrete it through bile due to the obstruction or congestion of the biliary tract. **Ahmed (32)** reported that, the increase in serum total bilirubin may be owing to blockage of bile ductules as the inflammation and fibrosis in the portal triads and/or due to the regurgitation of conjugated bilirubin from the necrotic hepatocytes to sinusoids. TAA might be due to the increased permeability of plasma membrane or cellular necrosis leading to leakage of the enzymes to the blood stream (33) and this showed the stress condition of the TAA treated animals. Also, the reduced cell viability in TAA treated hepatocytes may be due to total protein and albumin secretion outside the cells after TAA treatment confirmed the cell membrane damage and may be attributed to the effects of TAA on the liver either through inhibiting oxidative phosphorylation process and hence the availability of the energy source of protein synthesis. In addition, the markedly elevated bilirubin level may be due to liver cells are damaged and may be due to bile excretion is decrease by hepatotoxicity which drive to increase of bilirubin level due to body failure to excrete it through bile due to the congestion of the biliary tract.

The obtained results also showed that co-administration of M200 or M400 improved the liver function tests compared to TAA intoxicated rats. **Kumar (25) and (34)** reported that, the oral administration of methanolic extracts of *Origanum majorana* with CCL4 reduced direct bilirubin and total bilirubin levels. The extracts also improvement level of total protein significantly when compared to CCL4 treated group. The ethanolic extract of marjoram reform the levels of total protein, albumin and total bilirubin, thereby indicating their protection against the hepatotoxicity effects of TAA, which may be due to the inhibitory effects on cytochrome P450 resulting in the inhibition of formation of hepatotoxic free radicals.

The results of the present study also showed that, TAA administration caused a significant increase in serum TL, TC, TG and LDL concentration while HDL-C concentration was significantly the decreased comparing to control group. These results are in accordance with the previously reported results obtained by (35) and (36), who concluded that, TAA administration for eight weeks caused a significant increase in serum lipid parameters (TL, TC, TG and LDL-C) concentrations associated with significant decrease in HDL-C in rats intoxicated with CCl<sub>4</sub> comparing to control group. The increased of lipid profile may be due to the occurred liver disease as a result of deficiency of a functional liver which reflect the inability to metabolized lipid and hepatic damage leading to cell death. **McPhee and Hammer (37)** concluded that, most probably occurred in chronic liver diseases as a result of deficiency of a functional LDL which reflect the inability of the liver to clear LDL-C from the blood stream.

However, M200, M400 or sly administration with TAA showed a significant decrease in serum TL, TC, TG and LDL-C concentration associated with increased HDL-C concentration comparing to TAA intoxicated rats. The most beneficial effect was observed in M400+ TAA and sly +TAA groups. These findings are supported with (38), who reported that, *Origanum majorana* extract has a hyperlipidemic effect on lipopolysaccharide (LPS)-induced toxicity in rats indicated by significant decrease in the cholesterol and triglycerides levels. (39), who reported that, the chlorogenic acid and caffeic acid in *Origanum majorana* extract may act as hypocholesterolemic agent, reducing the LDL-c levels, when administrated orally to humans. These results are in the same line with those of (40) reported that, TC and TG were significantly decreased after feeding rat marjoram with cholesterol. The hypolipidaemic activity of M in rats resulted in could be attributed to the presence of valuable polyphenolic compounds, terpenoids, flavonoids, tannins, hydroquinone, phenolic glycosides and sabinene (41). The hypocholesterolemic effect of M could be attributed to presence of isoflavones which prevent intestinal absorption of cholesterol by competition for its absorption sites (42). These results are accordance (43), who found that marjoram extract lead to significantly lowering in TG than may be due lower fatty acids synthase.

The obtained data showed a significant increase in the hepatic peroxidation product (MDA) associated with significant decrease in the antioxidant markers (TAC, SOD, CAT, GSH and GST) in TAA intoxicated rats. These results are in agreement with (44) and (45), mentioned that, the level of liver MDA was significantly elevated in

TAA-treated rats and mice while SOD and GSH were decreased compared with the control group. **Mansour (46)** reported that, that TAA induced hepatic oxidative damage indicated by an increase in LPO and NO levels as well as the decrease in GSH content and SOD activity in experimental rats. **Saukkonen (47)** mentioned that , lipid peroxidation is involved in hepatic damage leading to cell death caused by an alteration in the intracellular prooxidant to antioxidant ratio in favor of prooxidants. Lipid peroxy radicals resulted in increased permeability of cell membrane, inactivation of membrane proteins and loss of polarity of mitochondrial membrane. These results may indicate an imbalance in free radical levels and hence increase in cellular damage. There is an imbalance between the amount of free radicals generated and the antioxidant present in the cell in favor of the oxidation **(48)**.

In case of rats co-administrated with M200 and M400 and TAA, there were a significant increase in hepatic TAC, SOD, GSH, GST and CAT associated with significant decrease in hepatic MDA comparing to TAA rat group. However, the hepatic TAC, SOD, GSH, GST and CAT in Sly+ TAA reaching to control normal level. The current results are in agreement with **(49)**, they mentioned that, co-administrated of rats with marjoram extract with CCL<sub>4</sub> showed a decrease in liver MDA and increase and liver TAC, CAT and SOD activities. Also, **(50) and (51)**, stated that, rats treated with marjoram extract showed a significant increase in liver SOD and GSH with significant decrease in liver MDA in paracetamol treated rats. The methanolic extract of M showed significant hydroxyl and superoxide radicals scavenging activity due to inhibition of respective mechanisms involved in their formation **(25)**. The administration of marjoram could reverse the TAA induced oxidative stress by scavenging the free radicals via the bioactive antioxidant constituents **(52)**. **Wahby (38)**, mentioned that, the ethanolic extract of *Origanum majorana* contain 3,4-dicaffeoylquinic acid, 4,5- dicaffeoylquinic acid, gallic acid, chlorogenic acid, tannic acid, quercetin, cinnamic acid and phloridzin as the polyphenols content. These polyphenols attenuated the decrease in antioxidant.

**Table1:-**Body weight (g) change, absolute (g) and relative liver weights (%) in control and different treated rat groups.

Animal groups Parameters	C	M200	M400	Sly	TAA	M200+ TAA	M400 TAA+	sly TAA+
Body Weight (g)	302.67 ±3.42 <sup>a</sup>	279.83 ±5.62 <sup>a</sup>	291.83 ±7.73 <sup>a</sup>	270.60 ±17.92 <sup>a</sup>	160.00 ±3.71 <sup>b</sup>	229.33 ±2.55 <sup>c</sup>	270.67 ±4.89 <sup>d</sup>	281.67 ±5.78 <sup>ad</sup>
Liver weight (g)	4.07 ± 0.30 <sup>a</sup>	3.74 ± 0.11 <sup>a</sup>	3.74 ±0.20 <sup>a</sup>	3.86 ±0.14 <sup>a</sup>	6.41 ±0.44 <sup>b</sup>	5.70 ±0.53 <sup>b</sup>	4.56 ±0.20 <sup>a</sup>	4.45 ±0.26 <sup>a</sup>
Relative liver weight (%)	1.34 ±0.08 <sup>a</sup>	1.34 0.04 <sup>a</sup> ±	1.28 ±0.07 <sup>a</sup>	1.44 ±0.08 <sup>a</sup>	4.01 ±0.05 <sup>b</sup>	2.49 0.08 <sup>b</sup> ±	1.69 0.07 <sup>c</sup> ±	1.58 0.1 <sup>a</sup> ±

-Each value represent the mean ± SE (n=6) and % of change (n=6 for each group)

-% of change compared to control group. Letters (a-c) express the significant change at p≤ 0.05. Similar letters (non-significant), different latters (significant)

-C: control M200: Marjoram extract 200mg M400: Marjoram extract 400mg TAA: Thioacetamide Sly: Silymarin.

**Table 2:-**Serum (AST), (ALT), (ALP) and (GGT) activities, Serum TP, albumin and total bilirubin levels in control and different treated rat groups.

Animal groups Parameter	Control	M200	M400	Sly	TAA	M200 + TAA	M400+ TAA	Sly TAA+
ALT (U/L)	34.11 ± 0.54 <sup>a</sup>	33.97 ±0.44 <sup>a</sup>	33.54 ±0.58 <sup>a</sup>	33.38 ±0.84 <sup>a</sup>	96.35 ±1.33 <sup>b</sup>	59.92 ±0.47 <sup>c</sup>	50.00 ±0.55 <sup>d</sup>	43.92 ±0.89 <sup>e</sup>
AST (U/L)	35.29 ±0.32 <sup>a</sup>	35.47 ±0.27 <sup>a</sup>	35.37 ±0.24 <sup>a</sup>	35.08 ±0.25 <sup>a</sup>	97.65 ±1.10 <sup>b</sup>	59.92 ±0.47 <sup>c</sup>	48.30 ±0.79 <sup>d</sup>	43.48 ±0.78 <sup>e</sup>
ALP (U/L)	44.62 ± 0.15 <sup>a</sup>	44.65 ±0.23 <sup>a</sup>	44.32 ±0.32 <sup>a</sup>	44.75 ±0.27 <sup>a</sup>	155.52 ±2.33 <sup>b</sup>	72.57 ±0.49 <sup>c</sup>	61.65 ±0.38 <sup>d</sup>	50.15 ±0.22 <sup>e</sup>
GGT (U/L)	19.41 ±0.24 <sup>a</sup>	19.42 ±0.22 <sup>a</sup>	19.35 ±0.17 <sup>a</sup>	19.15 ±0.23 <sup>a</sup>	40.33 ±1.30 <sup>b</sup>	29.28 ±0.35 <sup>c</sup>	25.07 ±0.33 <sup>d</sup>	20.97 ±0.17 <sup>a</sup>

Total protein (mg/dl)	6.63 ±0.07 <sup>a</sup>	6.44 ±0.11 <sup>a</sup>	6.62 ±0.07 <sup>a</sup>	6.62 ±0.03 <sup>a</sup>	3.52 ±0.10 <sup>b</sup>	5.28 ±0.12 <sup>a</sup>	6.55 ±0.15 <sup>a</sup>	6.24 ±0.12 <sup>a</sup>
Albumin (g/dl)	3.85 ±0.11 <sup>a</sup>	3.82 ±0.16 <sup>a</sup>	3.84 ±0.04 <sup>a</sup>	4.02 ±0.05 <sup>a</sup>	2.40 ±0.14 <sup>b</sup>	3.65 ±0.11 <sup>a</sup>	3.83 ±0.12 <sup>a</sup>	4.02 ±0.09 <sup>a</sup>
Bilirubin (mg/dl)	0.58 ±0.02 <sup>a</sup>	0.61 ±0.01 <sup>a</sup>	0.58 ±0.01 <sup>a</sup>	0.59 ±0.01 <sup>a</sup>	2.15 ±0.02 <sup>b</sup>	0.85 ±0.01 <sup>c</sup>	0.64 ±0.02 <sup>a</sup>	0.62 ±0.01 <sup>a</sup>

-Each value represent the mean ± SE (n=6) and % of change (n=6 for each group)

- % of change compared to control group. Letters (a-c) express the significant change at p≤ 0.05. -Similar letters (non-significant), different letters (significant) C: control M200: marjoram extract 200mg M400: Marjoram extract 400mg TAA: Thioacetamide Sly: Silymarin

**Table 3:-**Serum TL, TC, TG, HDL-C and LDL-C concentrations in control and different treated rat groups.

Animal groups parameters	Control	M200	M400	Sly	TAA	M200 TAA+	M400 TAA+	Sly TAA+
TL (mg/dl)	869.67 ±22.62 <sub>a</sub>	865.25 ±17.57 <sub>a</sub>	867.42 ±7.08 <sub>a</sub>	866.37 ±1.64 <sub>a</sub>	1104.00 ±17.16 <sub>b</sub>	954.50 ±7.67 <sub>c</sub>	887.25 ±10.17 <sub>a</sub>	858.00 ±3.14 <sub>a</sub>
TC (mg/dl)	58.50 ±0.94 <sub>a</sub>	58.12 ±1.68 <sub>a</sub>	58.77 ±1.99 <sub>a</sub>	58.65 ±2.01 <sub>a</sub>	99.35 ±0.41 <sub>b</sub>	72.32 ±1.76 <sub>c</sub>	63.00 ±0.57 <sub>a</sub>	62.57 ±0.93 <sub>a</sub>
TG (mg/dl)	47.54 ±1.91 <sub>a</sub>	47.16 ±1.52 <sub>a</sub>	47.25 ±0.66 <sub>a</sub>	47.15 ±1.98 <sub>a</sub>	88.67 ±0.27 <sub>b</sub>	69.27 ±1.21 <sub>c</sub>	50.75 ±0.22 <sub>a</sub>	46.50 ±0.70 <sub>a</sub>
HDL-C (mg/dl)	33.14 ±0.78 <sub>a</sub>	33.92 ±1.37 <sub>a</sub>	33.27 ±1.13 <sub>a</sub>	32.95 ±0.61 <sub>a</sub>	21.47 ±0.41 <sub>b</sub>	27.89 ±0.24 <sub>c</sub>	29.19 ±0.21 <sub>c</sub>	30.59 ±0.20 <sub>ac</sub>
LDL-C (mg/dl)	14.35 ±0.89 <sub>a</sub>	14.42 ±0.95 <sub>a</sub>	14.20 ±0.28 <sub>a</sub>	14.02 ±0.67 <sub>a</sub>	30.11 ±0.75 <sub>b</sub>	18.37 ±0.23 <sub>a</sub>	15.95 ±0.33 <sub>ac</sub>	15.20 ±0.11 <sub>c</sub>

-Each value represent the mean ± SE (n=6) and % of change (n=6 for each group).

-% of change compared to control group. Letters (a-c) express the significant change at p≤ 0.05. Similar latters (non-significant), different letters (significant).

-C: control M200: Marjoram extract 200mg M400: Marjoram extract 400mg TAA: Thioacetamide Sly: Silymarin

**Table 4:-**Hepatic total antioxidant capacity (TAC), superoxide dismutase (SOD) activity, reduced glutathione (GSH) content (m mol/g), glutathione-S-transferase (GST) activity, catalase (CAT) activity and malondialdehyde (MDA) content in control and different treated rat groups.

Animal groups Parameters	Control	M200	M400	Sly	TAA	TAA+ M200	TAA+M400	TAA+ Sly
TAC (mM/g)	4.47 ±0.07 <sub>a</sub>	4.36 ±0.06 <sub>a</sub>	4.43 ±0.09 <sub>a</sub>	4.36 ±0.03 <sub>a</sub>	3.16 ±0.06 <sub>b</sub>	3.66 ±0.06 <sub>c</sub>	4.12 ±0.04 <sub>d</sub>	4.32 ±0.02 <sub>ad</sub>
SOD (U/g)	958.23 ±16.10 <sub>a</sub>	970.41 ±5.81 <sub>a</sub>	954.00 ±11.40 <sub>a</sub>	946.50 ±12.97 <sub>a</sub>	638.17 ±47.76 <sub>b</sub>	782.25 ±1.70 <sub>c</sub>	878.92 ±10.07 <sub>d</sub>	945.00 ±4.58 <sub>a</sub>
GSH (m mol/g)	23.00 ±0.15 <sub>a</sub>	23.02 ±0.17 <sub>a</sub>	23.07 ±0.14 <sub>a</sub>	23.23 ±0.20 <sub>a</sub>	18.40 ±0.08 <sub>b</sub>	20.62 ±0.12 <sub>c</sub>	22.60 ±0.06 <sub>d</sub>	22.95 ±0.05 <sub>a</sub>
GST (m mol/g)	6.03 ±0.11 <sub>a</sub>	6.10 ±0.14 <sub>a</sub>	6.17 ±0.15 <sub>a</sub>	6.02 ±0.17 <sub>a</sub>	3.57 ±0.20 <sub>b</sub>	4.08 ±0.14 <sub>b</sub>	5.66 ±0.07 <sub>a</sub>	5.85 ±0.02 <sub>a</sub>
CAT (U/g)	2.70 ±0.09	2.41 ±0.03	2.45 ±0.12	2.66 ±0.14	1.22 ±0.02	2.01 ±0.02	2.24 ±0.06	2.32 ±0.07

	a	a	a	a	b	c	ac	a
MDA (nmol/g)	11.55 ±0.27 a	11.25 ±0.27 a	11.16 ±0.21 a	11.22 ±0.18 a	22.48 ±0.29 b	14.60 ±0.27 c	12.92 ±0.17 d	11.56 ±0.16 a

-Each value represent the mean  $\pm$  SE (n=6) and % of change (n=6 for each group).

-% of change compared to control group. Letters (a-c) express the significant change at  $p \leq 0.05$ . Similar latters (non-significant), different letters (significant).

-C: control M200: marjoram extract 200mg M400: marjoram extract 400mg TAA: Thioacetamide Sly: Silymarin

### Conclusion:-

The present data displayed the hepatoprotective effect of M400 and M200 with respect to Sly standard treatment. Such beneficial effects could be arranged in the following order: Sly > M400 > M200. Marjoram extract contains many antioxidant compounds, ranging from simple phenol to complex material as tannins which have the ability to inhibit the respective mechanisms involved in the formation of free radicals and enhancing the antioxidant status, they have also important beneficial effects on the liver regeneration.

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