Effect of *Calotropis Procera* extract on thioacetamide induced liver cirrhosis in rats

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A B S T R A C T

The present study was designed to evaluate the effect of *Calotropis procera* (CPA) latex treatment on liver cirrhosis induced in rats. Ninety male rats divided into 2 main groups. Group I: Comprised 10 rats served as control normal group injected with normal saline. Group II: included 80 rats injected with thioacetamide (TAA) (0.2 gm/kg b. wt, I. p), three times per week for 45 days. Group II was divided into 4 subgroups each 20 rats. Subgroup IIA: served as control liver cirrhosis non-treated group. Subgroup IIB: rats treated with silymarin at dose of 100 mg/kg / b .Wt /days orally for 60 days .Subgroup IIC: rats administered with CPA at dose of 200 mg/kg b.wt /day, orally for 60 days . Subgroup IID: rat's reserved silymarin at dose of 100 mg/kg b.wt/days and. CPA at dose of 200 mg/kg b.wt/ days orally for 60 days. Blood Samples and liver tissue specimens were collected after 30 and 60 day of treatment. Serum was separated and used directly for determents of Aspartate aminotransferase (AST), Alanine amino transferase (ALT), total protein (TP), albumin (ALB.), total bilirubin (T.Bili.), Alkaline phosphatase (ALP), Gamma glutamyl transaminase (GGT), total cholesterol (Chol.), triacylglycerides (TAG), high density lipoproteins cholesterol (HDL-C) and Interleukin-8 (IL-8), Moreover, glutathione-S-transferase (GST), Catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH)were determined liver tissues ,furthermor, liver histopathology were also investigated . The results revealed that, a significant increase in serum AST, ALT, ALP, T. Bili., Serum GGT, IL8, Chol. And TAG with significant decrease of TP, ALB. , HDL-C, enzymatic antioxidant states (GST, CAT, SOD) and (GSH), in liver tissue were observed in thioacetamide injected groups of rates when compared to the control group. However, treatment with silymarin and C. *procera* only and their combination improved liver functions test parameters, and aim of the work these parameters to normal level. Recommended to use medicinal herbal plant in treatment of liver toxicity.

Keywords: antioxidant enzymes, liver enzymes, *Calotropis procera* latex extract and silymarin.

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1. INTRODUCTION

Thioacetamide is a potent experimental hepatotoxin and hepato-carcinogenic compound; therefore, it used often to induce fulminant hepatic failure (FHF) in experimental animal models (Sarker, and Sil, (2007). Thioacetamide induces hepatotoxicity via its S-oxide metabolite (thioacetamide-S-dioxide), an unstable, reactive metabolite, which initiates necrosis and the generation of reactive oxygen species (ROS) by binding covalently to liver macromolecules (Baskaran, et al. 2010). Also, TAA induces hepatocyte damage following its metabolism to thioacetamide sulphene and sulphone, via a critical pathway that involves CYP4502E1-mediated biotransformation (Ramaiah, et al., 2001). Medicinal plants play a key role in the human health care. Natural products, especially plants in folk medicine with an anecdotal history of positive effects against liver diseases or other organs, are considered an alternative therapeutic approach (Khanna et al., 2007). Due to lack of scientific-based pharmacological data, most of the herbal formulations cannot be recommended for the

treatment of liver diseases (Stickel and Schuppan, 2007). Hence, there is an ever increasing need for safe hepatoprotective agent (Agar-wal, 2001). Silymarin is the main active constituent in the seeds of Silybum marianum, or milk thistle, Silybum marianum L.. Sylibum marianum has antioxidative, antilipid peroxidative, antifibrotic, anti-inflammatory, immunomodulating and liver regenerative (Thyagarajan et al., 2002). In addition, the hepatoprotective action of silymarin is mainly through its anti-free radical and anticarcinogenic roles Shaker et al., (2010). The activity of silymarin is due to its constituent silybin, silydianin, and silychristine (Loguercio and Festi, 2011). Calotropis procera (Ait.) R. Br. is a wild growing tropical plant of family Asclepiadaceae that has been widely used in the Sudanese, Unani, Arabic and Indian traditional medicinal system for the treatment of various diseases namely leprosy, ulcers, tumors, piles and diseases of the spleen, liver and abdomen (Kumar and Arya, 2006). Some of the medicinal properties of this plant have also been demonstrated in acute and sub-acute animal studies using either the aerial parts of the plant, its roots or the latex (Kumar and Arya, 2006). The latex of the plant that occurs in abundance in its aerial parts has been shown to produce antidiabetic, hepatoprotective, antiarthritic, cytotoxic and anti-cancer effects (Padhy, et al., 2007).

2. Materials and Methods

2.1.Experimental animal rats;

Ninety male Sprague Dawely rats of8-10weeks old and weighting 150 to 200g. Rats were obtained from the Research Institutes of Ophthalmology, Giza, Egypt. Animals were housed in separate metal cages, fresh and clean drinking water was supplied ad-libtium. Rats were kept at a constant environmental and nutritional condition throughout the period of the experiment.

2.2.Extraction of latex of Calotropis procera:

Fresh latex of Calotropis procera plant were collected from the aerial parts of the plant growing in the wild was air dried under shade at ambient temperature with a yield of 20 gm per 100 ml and ground to small granules (DL). The DL was triturated with gum acacia (1:1) in normal saline to obtain an aqueous suspension. The filtrate served as the stock solution. The herbal extract was given orally and daily at a dose of 200 mg/Kg b.wt, for 60 days (Singhal, A. and Kumar, V. L. 2009).

2.3.Experimental design

Rats were divided into 2 main groups: Group I: Consists of 10 rats injected with normal saline and saved as control. Group II: included 80 rats injected with TAA (0.2 gm/kg b.wt i.p), three times per week for 45 days. This group was divided into 4 equal subgroups each consist of 20 rats: Subgroup II a: rats received no drags and acting as control non treated group. Subgroup IIb: rats treated with silymarin at dose of 100 mg/kg b.wt /day orally for 60 days. Subgroup IIc: rats administered with CPA at dose of 200 mg/kg b.wt/ day orally for 60 days. Subgroup IId: rats administered with silymarin at dose of 100 mg/kg.b.wt and. CPA at dose of 200 mg/kg.b.wt/ days orally for 60 days.

2.4.5-Sampling:

Blood samples and liver tissues were collected after 30 days and 60 days from the onset of beginning treatment. A-Blood samples were collected into clean, dry centrifuge tubes, left at room temperature for 15 minutes to clot, centrifuged at 1000g for 10 minutes for serum separation. Serum was carefully aspirated and transferred into dry clean Eppindorf tubes using Pasteur pipette then kept in a deep furze at -20c^0 until used four subsequent biochemical analyses. biochemical analyses; Serum ALT, AST, ALP and GGT activities ,and T.Bili TP. ALB. T.Chol., TAG. HDL-C IL-8 concentration were determined according to

the methods described by Pappas ., 1989, Tietz, N. W. 1995,Vázquez-Medina et al., 2011, Malloy and Evolyn, 1937, Josephson., 1957, Young et al., 1995, Lee et al., 2008, (ELISA) kit supplied by Cortez Diagnostics , the supernatant of liver tissue homogenate were used for the determination of (SOD, CATand GST) activities, GSH concentration according to the methods described by(Nishikimi, et al., 1972) , (Abei , 1984), (Habig and Pabst, (1974), (Beutler, et al., 1963) respectively. b-Liver spsmen : rat's sacrified for removal of liver tissues. The liver divided into two parts: 1-four biochemical analysis. Measuring of GST (Habig and Pabst, (1974), CAT (Abei, (1984), SOD (Nishikimi, et al., 1972) and GSH (Beutler, et al., 1963). The liver spesmen was perfused with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg / ml heparin to remove any red blood cells and clots. Then, liver was weighed, minced, homogenized in 5 – 10 ml cold buffer (i.e. 50 mM potassium phosphate, pH 7.5. 1 mM EDTA) per gm liver tissue .Homogenates were centrifuged at 10,000 rpm for 20 minutes at 4°C and the supernatant was kept at −20 ◦C till used for analysis of antioxidant enzymes. 2-four Hisopathology findings: The secand part was kept in formalin 10% for histopathology examenation.

2.5.Statistical analysis:

Results were expressed as Means ± standard deviation (SD). Statistical analysis was done by using SPSS computer program (version 10). The significance of differences was calculated by using ONE WAY ANOVA with Tukey's posthoc test, $p < 0.05$ was considered statistically significant.

3. RESULTS:

The results presented in (Tables 1, 2) revealed that, rats injected with TAA-induced cirrhosis caused significant increase in serum ALT, AST, ALP and GGT activities and T. Bili Level after 30 and 60 days when compared with control group. Meanwhile, a significant decrease in serum total protein and albumin level were observed in TAA injected rats. Treatment with silymarin and *C. procera* only and their combination lowered the elevated levels of AST, ALT, ALP and GGT activities and T. Bili level.; while, total protein and albumin non significantly increased when compared to the TAA treated group. The data prefect in table 3 and 4 showed a significant increase in serum TAG, T. Chol with significant decrease in HDL-C in TAA injected rats after 30 and 60 days as compared with control. Treatment of TTA induced cirrhotic rats with silymarin or/and *C. procera* caused a significantly decrease in serum T.Chol. And TAG concentration and significantly increased serum HDL-C in 30 and 60 days when compared with thioacetamide treated group. The results presented in (Table, 3 and 4) reveled That a significant increase in serum IL-8 concentration was observed in rats injected with TTA after 30 and 60 days when compared with control group. Treatment with silymarin or/and *C. procera* showed significant decrease in serum IL-8 level after 30 and 60 days when compared to thioacetamid non treated group. The results printed in (Tables 5and 6) showed that, in TTA injection to rats caused significant decrease in the activity of the liver antioxidant enzymes GST, CAT, SOD and GSH concentration after 30 and 60 days when compared with control. Treatment of TTA intoxicated rats with silymarin or/and *C. procera* caused a significant increase in the activity of the antioxidant enzymes GST, CAT, SOD and GSH concentration after 30 and 60 days as compared with TTA non treated group. Histopathological finding in (fig., 2, 3, 4, 5, 6, 7, 8 and 9) showed liver cirrhosis and marked portal tracts fibrosis in thioacetamide injected group when compared to control. Treatment of TTA intoxicated rats with silymarin or/and *C. procera* caused improvement in liver fibrosis with regeneration of hepatocytes became nearly similar to normal liver.

Table (1): Effect of treatment with Silymarin and Calotropis Procera alone or Their combination on serum ALT, AST, ALP and GGT activities and total bilirubin, Total protein and Albumen concentration in experimentally induced liver cirrhosis in rats after 30 days

Animal group	ALT (U/L)	AST(U/L)	ALP(U/L)	GGT (U/L)	total bilirubin (mg/dl)	Total protein (g/dl)	Albumen (g/dl)
Control group	40.25 ± 2.87	74.25±6.87	216.50± 7.80	22.25± 3.30	0.14±0.05	10.30± 0.22	4.20 ± 0.38
Thioacetamide group	73.50±6.97^{A}	380.75±12.23^{A}	423.75 ± 13.62^{A}	87.75± 7.89^{A}	0.20± 0.08^{A}	8.00± 0.42^{A}	3.35± 0.25^{A}
Silymarin group	50.00± 1.41^{AB}	322.oo±8.86^{ABC}	345.00 ± 9.13^{AB}	31.25± 2.22^{AB}	0.17 ±0.06	8.95 ± 0.21^{A}	4.03 ± 0.27
CPA group	45.50±4.50^{AB}	357.50±11.86^{AB} C	365.25±8.55^{ABC}	25.75± 3.86^{B}	0.14 ± 0.06^{B}	9.85 ± 0.31^{B}	3.83 ± 0.46
Silymarin + CPA group	42.75±3.95^{AB} C	193.50±9.35^{ABC}	375.00±6.91^{ABC} D	34.00± 6.48^{ABD}	0.12±0.06^{BCD}	9.68 ± 0.50^{B}	3.65 ± 0.57

All data were expressed as mean ± SD. One way annova test with Scheffe's posthoc test. ^{A} Significant vs saline group, ^{B} Significant vs thioacetamide group ^{C} Significant vs thioacetamide+ sylmarin group ^{D} Significant vs thioacetamide + CPA group ($p \leq 0.05$). ^{E} significant vs the same group at 30 days.

Table (2): Effect of treatment with Silymarin and Calotropis Procera alone or Their combination on serum ALT, AST, ALP and GGT activities and total bilirubin, Total protein and Albumen concentration in experimentally induced liver cirrhosis in rats after 60 days

Animal group	ALT (U/L)	AST(U/L)	ALP(U/L)	GGT (U/L)	total bilirubin (mg/dl)	Total protein (g/dl)	Albumen (g/dl)
Control group	37.50± 2.89	69.26±8.46	217.75 ± 6.56	22.75± 1.99	0.13±0.02	9.88 ± 0.31	4.25 ± 0.31
Thioacetamide group	58.00±5.23^{AE}	143.75±8.54^{AE}	283.50±7.92^{AE}	63.25± 6.45^{AE}	0.38± 0.17^{AE}	6.25±0.81^{AE}	2.50±0.29^{AE}
Silymarin group	41.50± 6.60^{BE}	135.00±6.24^{AB}	174.50±7.26^{AB}	28.75± 2.12^{AB}	0.24 ± 0.05^{AB}	7.53 ± 0.25^{B}	3.78± 0.43^{B}
CPA group	48.oo±2.49^{AB}	149.00±7.70^{ABCE}	284.00±9.17^{ABCE}	29.50± 2.80^{ABC}	0.19 ± 0.02^{B}	7.15 ± 0.33^{B}	2.88 ± 0.41
Silymarin + CPA group	36.50±1.29^{ABCD}	135.50±7.45^{ABCDE}	193.25± 8.86^{ABCD}	19.50±1.29^{ABCD}	0.19 ± 0.08^{B}	7.65 ± 0.38^{B}	3.83± 0.49^{B}

All data were expressed as mean ± SD. One way annova test with Scheffe's posthoc test. [A] Significant vs saline group, [B] Significant vs thioacetamide group [C] Significant vs thioacetamide+ sylmarin group [D] Significant vs thioacetamide + CPA group ($p \leq 0.05$). [E] significant vs the same group at 60 days.

Table (3): Effect of treatment with Silymarin and Calotropis Procera alon or Their combination on serum total cholesterol (Chol.), triacylglycerides (TAG), high density lipoproteins cholesterol (HDL-C) and Interleukin-8 (IL-8) in experimentally induced liver cirrhosis in rats (after 30 day).

Animal group	Total(Cho.) mg/dl	TAG mg/dl	HDL.C mg/dl	IL-8 pg/ml
Control group	98.25 ± 3.50	73.50 ±4.89	27.25±2.19	2.56 ± 0.17
Thioacetamide group	110.25±2.75^{A}	78.25 ± 3.47^{A}	15.25 ± 2.63^{A}	39.61 ± 1.56^{A}
Silymarin group	89.25 ±7.06^{B}	77.50 ± 3.14^{AB}	23.25± 1.71^{B}	18.15 ± 0.45^{AB}
CPA group	98.25 ± 4.73^{B}	64.25 ± 2.87^{ABC}	23.50±1.65^{B}	2.24 ± 0.34^{ABC}
Silymarin + CPA group	81.75 ± 6.43^{B}	61.00 ± 2.68^{ABC}	21.00 ± 2.82^{B}	9.42 ±0.28^{ABCD}

Table (4): Effect of treatment with Silymarin and Calotropis Procera alon or Their combination on serum total cholesterol (Chol.), triacylglycerides (TAG), high density lipoproteins cholesterol (HDL-C) and Interleukin-8 (IL-8) in experimentally induced liver cirrhosis in rats (after 60day).

Animal group	Total (Cho.) mg/dl	TAG mg/dl	HDL.C mg/dl	IL-8 pg/ml
Control group	94.25± 4.90	79.25± 4.63	26.25 ± 1.95	3.19 ± 0.21
Thioacetamide group	100.00±2.76^{AE}	94.75 ± 3.81^{AE}	14.75 ± 1.50^{AE}	51.67 ± 2.68^{AE}
Silymarin group	62.50 ± 1.07^{AB}	106.50 ± 2.76^{AB}	23.00 ± 2.35^{B}	16.34 ± 0.26^{AB}
CPA group	56.00 ± 2.06^{ABE}	60.00 ± 2.38^{ABC}	23.75 ± 1.59^{BE}	9.82 ± 0.14^{ABCE}
Silymarin + CPA group	80.75 ± 3.53^{AB}	59.50±3.93^{ABC}	21.25 ± 2.64^{B}	7.43 ± 0.31^{ABCD}

All data were expressed as mean ± SD. One way annova test with Scheffe's posthoc test. ^{A} Significant vs saline group, ^{B} Significant vs thioacetamide group ^{C} Significant vs thioacetamide+ sylmarin group ^{D} Significant vs thioacetamide + CPA group ($p \leq 0.05$). ^{E} significant vs the same group at 30 and 60 days.

Table (5): Effect of treatment with Silymarin and Calotropis Procera alon or Their combination on liver tissue (GST, CAT and SOD) activities and GSH concentration in experimentally induced liver cirrhosis in rats. (After 30 day)

Animal group	*SOD* (u/g tissue)	*Catalase* (u/g tissue)	*G-S-T* (u/g tissue)	*Reduced glutathione* (mg/g. tissue)
Control group	252.25±8.01	0.71 ± 0.13	3.32 ± 0.18	3.30 ± 0.12
Thioacetamide group	163.42±7.99^{A}	0.19 ± 0.04^{A}	1.93 ± 0.20^{A}	1.98 ± 0.14^{A}
Silymarin group	233.75± 8.32^{AB}	0.55± 0.17^{B}	3.11±0.19^{B}	2.95 ± 0.47^{B}
CPA group	258.25 ± 7.99^{AB}	0.60 ± 0.06^{B}	2.65 ± 0.19^{B}	3.22 ± 0.08^{B}
Silymarin + CPA group	237.75± 9.70^{BCD}	0.75± 0.06^{BC}	2.97 ± 0.38^{B}	3.20 ± 0.17^{B}

Table 6. Effect of treatment with Silymarin and Calotropis Procera alon or Their combination on liver tissue (GST, CAT and SOD) activities and GSH concentration in experimentally induced liver cirrhosis in rats. (60 days)

Animal group	*SOD* (u/g tissue)	*Catalase* (u/g tissue)	*G-S-T* (u/g tissue)	*Reduced glutathione* (mg/g. tissue)
Control group	248.75± 4.67	0.76 ± 0.11	3.25 ± 0.15	3.22 ± 0.11
Thioacetamide group	132.00±6.96^{AE}	0.16± 0.03^{AE}	1.82 ± 0.11^{A}	1.95± 0.15^{A}
Silymarin group	180.25± 7.99^{ABE}	0.41 ± 0.15^{B}	2.48±0.27^{BA}	2.97 ± 0.14^{B}
CPA group	185.00± 6.81^{ABE}	0.50 ± 0.04^{B}	2.30 ± 0.14^{B}	2.68 ± 0.12^{B}
Silymarin+c CPA group	174.75±4.21^{ABCDE}	0.52 ± 0.07^{B}	2.51 ± 0.17^{B}	3.13 ± 0.17^{B}

All data were expressed as mean ± SD. One-way annova test with Scheffe's posthoc test. ^{A} Significant vs saline group, ^{B} Significant vs thioacetamide group ^{C} Significant vs thioacetamide+ sylmarin group ^{D} Significant vs thioacetamide + CPA group ($p \leq 0.05$). ^{E} significant vs the same group at 30 and 60 days.

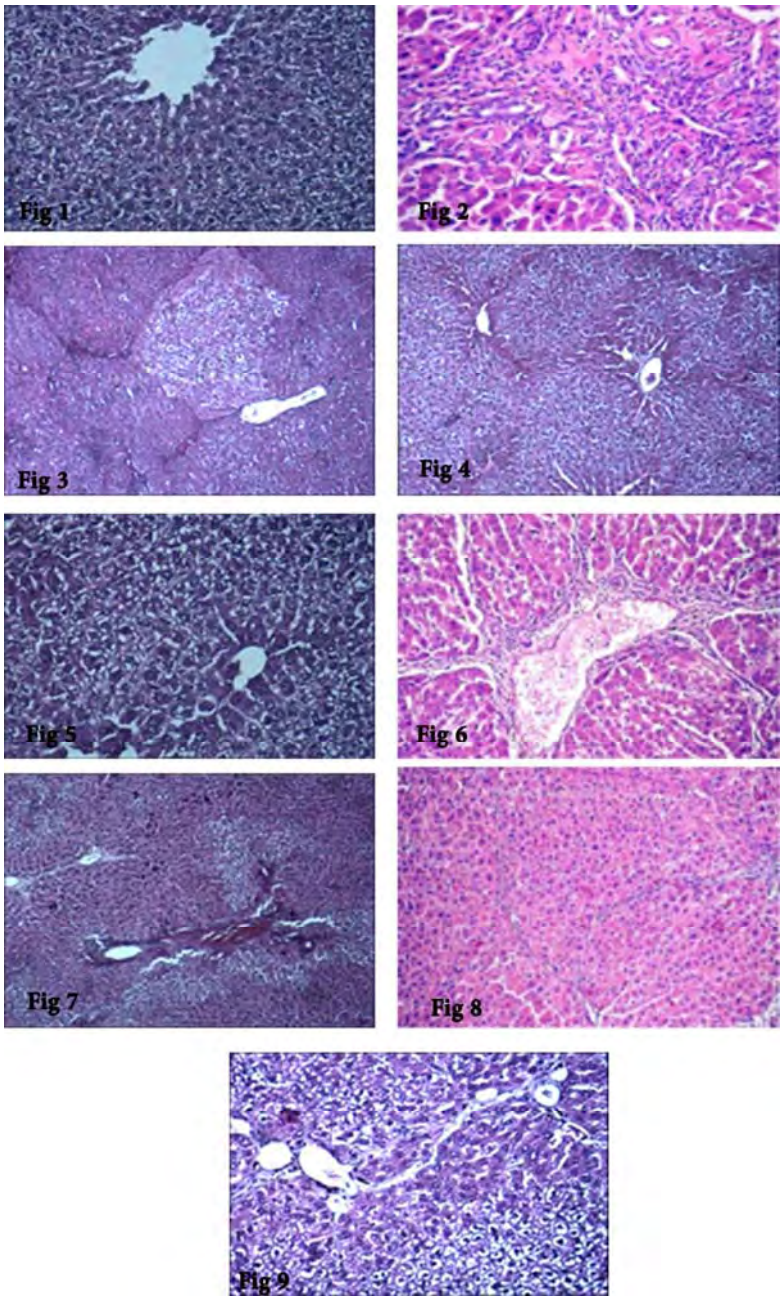

Fig. (1): liver specimen of normal liver showing normal hepatocytes and normal portal tracts with no evidence of fibrosis (Saline control group) H and E 200x. Fig. (2): liver specimen of cirrhotic liver showing liver cirrhosis and marked portal tracts fibrosis (Thioacetamid treated group after (30 day) H and E 200x. Fig. (3): liver specimen of cirrhotic liver showing cirrhotic nodules with portal tracts fibrosis and inflammatory infiltrate (Thioacetamide treated group after (60dayes) H and E 200x. Fig. (4): Liver specimen of cirrhotic liver showing regenerating hepatocytes starting the periphery (Sylmarin treated group after (30days) H and E 200x. Fig. (5): Liver specimen of cirrhotic liver showing marked regeneration of hepatic lobules (Sylmarin treated group after group 60day) H and E 200x. Fig. (6): Liver specimen of cirrhotic liver showing marked regeneration of hepatic lobules (CPA group30day) H and E 200x. Fig. (7): Liver specimen of cirrhotic liver showing marked regeneration of hepatic lobules with mild fibrosis of portal tracts (CPA group60dayes) H and E 200x. Fig. (8): Liver specimen of cirrhotic liver showing marked regeneration of hepatic lobules with no evidence fibrosis of portal tracts (CPA+ sylmarin group30days) H and E 200x. Fig. (9): Liver specimen of liver showing marked regeneration of hepatic lobules with no normal portal tracts (Near normal liver) (CPA+ sylmarin grou60dayes p) H and E 200x

4. DISCUSSION

Liver cirrhosis is an irreversible process characterized by excess extracellular matrix (ECM) deposition in the liver accompanied with scar formation and destruction of the liver architecture (Pinzani, 2000). In the present study, silymarin and C. procera were examined for treatment of liver cirrhosis and significant increase in serum ALT, AST, ALP and GGT activities and T. Bili level were observed in rats injected with TTA when compared with control. This obtained result was nearly similar to the previously reported data by (Kumar, et al., 2007). Also, results come in accordance with (Baskaran, et al. 2010) who recorded that; thioacetamide induces hepatotoxicity via its S-oxide metabolite (thioacetamide-S-dioxide), an unstable and reactive metabolite, which initiates necrosis and the generation of *ROS* by binding covalently to liver macromolecules. Moreover, Chu, et al. (2000**)** demonstrated that, thioacetamide (TAA) is a hepatotoxin causing centrilobular necrosis which has been shown to also induce apoptosis and periportal inflammatory cell infiltration in rat liver. Treatment with silymarin and C. procera alone and their combination lowered the elevated levels of AST, ALT, ALP, GGT activities and T. Bili level In rats treated with TAA. These findings were in agreement with **(**Chavda, et al., 2010) who showed that treatment of rats with silymarin or/and C. procera significantly lowered the elevated levels of AST, ALT, ALP, GGT activities and T. Bili level in rats treated with TAA. Who concluded its hepatoprotective potential. Also, Ramachandra, et al., (2007) reported the hepatoprotective effect of C. procera flowers. Injection of thioacetamide to normal rats exhibited a significant decrease in serum total protein and albumin concentration when compared with control. These results were in agreement with (Rao, et al., 2014) who reported that, a decrease in serum level of total proteins and albumin were observed in TAA-treated rats, suggests the severity of liver toxicity. This alteration could be related to the induction of abiquitin-associated protein degradation by TAA toxic stress (Andersen, et al., 1981). Treatment of TTA intoxicated rats with silymarin or/and C. procera resulted in a non significance increase in serum total protein and albumin level as compared to the TAA treated group. The previous results were in accordance with (Chavda, et al., 2010) who reported that, administration of silymarin or/and C. procera significantly increased the value of serum of total protein and albumin concentration in rats with hepatotoxicity compared to the control group. In the current study marked increase in the concentrations of serum total Chol. and TAG. with a decrease of HDL-C level were observed in the TAA treated group when compared with control group, which may reflect the impairment of liver function, particularly on lipid metabolism. These results are in agreement with the reported data of (Ahmed, et al., 2012) who found that TAA injected rats strongly showed significant elevation of total Chol., TAG. and LDL-C levels with a decrease in the level of HDL-C as compared to control group. These findings could be due to reduction of formation of hepatic lipids. Inhibition of lipogenesis is transcriptionally activated by membrane bound transcription factors, such as sterol regulatory element-binding protein-1 (SREBP-1) (Foretz, et al., 1999).On the other hand, (Kumar, et al., 2008) reported that, there was no significant rise in total cholesterol and triacylglycrol levels were observed in thioacetamide treated group. The present results reported in (Tables 3and 4), raveled that treatment of TTA intoxicated rats with silymarin or/and C. procera caused a significant decrease in total Chol and TAG. concentrations are significant increase in HDL-C when compared with thioacetamide non treated

group. These results are in agreement with their recorded by (Salama, et al., 2012) who reported that, administration of silymarin or/and C. procera to rats with impaired lipid profile consequences in significant reduction in LDL, VLDL, triglyceride and cholesterol with elevation of HDL-C concentrations. Fatima, et al., (2013) discussed that, Silymarin works as anti-lipid peroxidation, in detoxification reactions, reduces leukotiene synthesis from unsaturated free acids, increases synthesis of proteins, stabilizes mast cells and also function in the regulation of immune system. However, (Kumar, et al., 2013) reported that, the extract of C. procera provided protection against iron induced lipid peroxidation in rat tissue (liver, brain, and kidney) homogenates. In the present study, injection of TAA intraperitoneally to rats resulted in a significant increase in serumIL-8 level when compared with control group. These are in agreement with (Amanzada, et al., 2014) who recorded that, an early increase in serum IL-8 level was observed after TAA administration in rats when compared with control group. Interleukin-8 may act as a good diagnostic and prognostic parameter in evaluating the severity of liver damage (Dashti, et al., 1996). One of the key proinflammatory cytokines involved in modulating the inflammatory response is the CXC chemokine IL-8, which functions as a critical chemo attractant and activator for neutrophils, basophils, and T cells **(**Remick, 2005). After treatment with silymarin or/and C. procera a significant decrease in IL-8 level were observed at the same intervals of follow up when compared with thioacetamide group. Unfortunately, there are no previous studies about the effect of the herbal drugs on the plasma levels of IL-8; but (Padhy, et al., 2007) stated that, daily oral administration of aqueous suspension of dried latex (DL) of Calotropis procera in rats intoxicated with carbontetracholride (CCl4) produced a marked elevation in the serum levels of the pro-inflammatory cytokine (TNF-α).Intraperitoneal injected of TTA in normal rats exhibited a significant decrease in the activity of the antioxidant enzymes(GST, CAT and SOD) activities and GSH concentration as compared to normal control. These are in agreement with (Cruz, et al., 2005) who recorded that TTA significantly decreased the activity of the antioxidant enzymes (GST, CAT and SOD) activities and GSH concentration. Uskokovic-Markovic, et al., (2007) recorded that, thioacetamide causes an elevation of oxidative stress, enhancing free radical– mediated damage to proteins, lipids and deoxyribonucleic acid (DNA). In present study treatment of TTA intoxicated rats with silymarin or/and C. procera caused a significant increase in the activity of the antioxidant enzymes GST, CAT, SOD and GSH level compared with control. These results are in agreement with (Amin, et al., 2012; Padhy, et al., 2007) who stated that, treatment of TTA intoxicated rats with silymarin or/and C. procera caused a significant increase in the activity of the antioxidant enzymes. Also, Ghosh, et al., (2010**)** reported that, Silymarin appears to act as an antioxidant not only because it acts as a scavenger of the free radicals that induce lipid peroxidation, but also because it influences enzyme systems associated with glutathione and superoxide dismutase. The obtained results for the antioxidant enzymes suggest that, the hepatoprotective effect of the extracts may be due to C. procera contains terpenoids (Sunitha, et al., 2001) and flavanoids (Janbaz, et al., 2002) which might have scavenged the free radical offering hepatoprotection. The antioxidant property of DL has been attributed to its constituients cardinolides, lignans and flavanol glycosides (Roy, et al., 2005). Histologically, in TAA injected rats the specimen of liver showed cirrhosis and marked portal tracts fibrosis compared with control group. These results came in agreement with (Ahmed, et al., 2012**),**who recorded that, the liver sections of TAA-treated animals showed hepatic cells with severe toxicity characterized by centrilobular necrosis, preiportal hepatocyte

vacoulation with clearing of cytoplasm, scattered inflammation and cell transformation .Treatment of rats with silymarin or/and C. procera , reversed TAA-induced pathogenic changes in liver caused improvement in liver fibrosis with regeneration of hepatocytes became nearly similar to normal liver. These results are in accordance with (Fatima, et al., 2013) who recorded that, reduction in the amount of fibrous tissue and the stage of nodule formation was inhibited the architecture of liver tissue is maintained in rats treated with Silymarin compared to TAA treated group. Also, Choedon, et al., (2006) recorded that, the aqueous extract of Calotropis procera has been shown to inhibit cellular infiltration and afford protection against development of neoplastic changes in the transgenic mouse model of hepatocellular carcinoma. Moreover**,** Singhal and Kumar, (2009) reported that, histological finding of liver and kidney of Calotropis procera DL treated rats did not show any pathological changes. The results of the present investigation indicate that the the latex of Calotropis procera affords good results against TAA induced hepatotoxicity, have considerable potential to chelate metal ions as well as scavenge free radicals generated in the system and thereby neutralize metal ion mediated oxidative damage. Therefore, C. procera latex could be regarded as a source of future antioxidant compounds of natural origin we advise with further investigations required to characterize the active hepatoprotective principle and its mechanism of action.

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التأثير الوقائي لمستخلص نبات العشار في تليف الكبد المحدث بالثيواسيتاميد في الجرذان

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الملخص العربى

تليف الكبد هو أحد الأمراض المزمنة الأكثر انتشارا فى العالم ويرتبط مع معدلات الاعتلال والوفيات ارتباطا كبيرا، ومن ثم فإن ايجاد دواء كبد فعال هو حاجة ملحة. وقد أجريت هذه الدراسة لتقييم ما إذا ما كان نبات العشار له القدرة على منع التليف الكبدى الناجم عن التسمم بالثيواسيتاميد في الفئران. وقد أجريت هذه الدراسة على عدد تسعون من ذكور الجرذان البيضاء والتي تتراوح أعمارهم من 8 -10 اسابيع وأوزانهم من 150 -200 جرام، وقد تم تقسيمهم إلى مجموعتين: المجموعة الأولى (المجموعة الضابطة): وهي المجموعة القياسية وتتكون من10جرذان وتم حقنها بـ 0.2جرام لكل كجم من محلول الملح ثلاث مرات من كل أسبوع. المجموعة الثانية (مجموعة الثيواسيتاميد): اشتملت على 80 من الجرذان وقد أعطيت المستخلص الثيواسيتاميد عن طريق الحقن بـ 0.2 جرام لكل كجم ثلاث مرات من كل أسبوع لمدة 7 أسابيع لإحداث التليف الكبدي. بعد 45 يوم تم تقسيم المجموعة الثانية إلى 4 مجموعات فرعية متساوية اشتملت كل مجموعة على 20 جرذا: المجموعة الفرعية الأولى: استخدمت كمجموعة ضابطة لم تعطى أي دواء. المجموعة الفرعية الثانية: تم تجريع الجرذان عن طريق الفم بالسيلمارين كجرعة مقدارها 100 مليجرام لكل كجم مرة يوميا لمدة 60 يوم. المجموعة الفرعية الثالثة : تم تجريع الجرذان عن طريق الفم بمستخلص نبات العشار جرعة مقدارها 200 مليجرام لكل كجم مرة يوميا لمدة 60 يوم. المجموعة الفرعية الرابعة: تم تجريع الجرذان عن طريق الفم بالسيلمارين جرعة مقدارها 100 مليجرام لكل كجم مع مستخلص نبات العشار بجرعة مقدارها 200 مليجرام جرعة لكل كجم مرة يوميا لمدة 60 يوم. اسفرت الدراسة التي أجريت على ذكورالجرذان المحدث فيها التليف الكبدي بالثيواسيتاميدعن وجود زيادة نشاط انزيمات الكبد و البيليروبن الكلى زيادة معنوية، كذلك حدثت زيادة معنوية فى انترليوكين8، الكوليسترول الكلى، الدهون الثلاثية في مصل الجرذان. بينما كان هناك نقص معنوى فى مستوى البروتين الكلى والألبومين والكوليسترول عالي الكثافة ونقص فى نشاط الأنزيمات المضادة للأكسدة مثل إنزيم السوبراكسيد ديسميوتيز، الكتاليز، الجلوتاثيون المختزل و إنزيم جلوتاثيون بيروكسيديز فى ذكورالجرزان المحدث لها التسمم الكبدي بالثيواسيتاميد عند مقارنتها بمجموعة الجرذان الطبيعية. بينما أسفرت الدراسة انه عند علاج ذكورالجرذان المحدث فيها التليف الكبدي بالثيواسيتاميد بالسليمارين أو مستخلص نبات العشار أوكلاهما معا عن وجود نقص معنوى فى نشاط انزيمات الكبد و البيليروبن الكلى، كذلك حدث نقص معنوى فى انترليوكين8، الكوليسترول الكلى، الدهون الثلاثية في مصل الجرذان . بينما حدث زيادة معنوية فى نشاط كلا من:- الأنزيمات المضادة للأكسدة مثل إنزيم السوبراكسيد ديسميوتيز، الكتاليز، الجلوتاثيون المختزل، إنزيم جلوتاثيون بيروكسيديز وأيضا زيادة معنوية فى مستوى البروتين الكلى، الألبيومين و الكوليسترول عالى الكثافة فى مجموعات الجرذان المعالجة بالسليمارين أو مستخلص نبات العشار أوكلاهما معا والمحدث لها التسمم الكبدي بالثيواسيتاميد عند مقارنتها بمجموعة الثيواسيتاميد فقط. و قد خلصت النتائج الى أن السيليمارين ونبات العشار منع موت الخلايا و التليف الكبدى الواضح الناجم عن الثيواسيتاميد فى الأنسجة الكبدية مقارنة بمجموعة الجرذان الطبيعية . و قد أسفرت نتائج الدراسة أيضاعن حدوث تحسن واضح فى وظائف الكبد و أنسجته نتيجة المعالجة بالسليمارين أو مستخلص نبات العشار أوكلاهما معا من خلال إحداث انخفاض كبير في مستويات الشوارد الحرة والزيادة في مضادات الأكسدة.

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