

## HEPATOPROTECTIVE EFFECTS OF TURMERIC AND MILK THISTLE SEED FLOUR AGAINST ETHANOL LIVER DAMAGE IN WISTER RATS

F. M. El-Shouny, S. K. El-Kadousy, S. M. El-Gawad and Kh. A. El-Khalwy  
Biochemistry- Dep., Faculty of Agricultural, Menoufia University

Received: Oct. 31 , 2021

Accepted: Nov. 16 , 2021

**ABSTRACT:** Chemical composition of milk thistle seed flour (MTSF) and turmeric (T) were investigated, for milk thistle seed flour, moisture (8.0%), protein (13.0 %), carbohydrates (27.55%), crude lipid (15.0%), ash (10.45%), and crude fiber (26.0%), while turmeric has moisture (8.1%) , protein (9.3 %), carbohydrates (57%), crude lipid (17.0%), ash (6.0%) and crude fiber (2.6%). For total phenol, the highest value in turmeric (11.57mg GAE/g) while milk thistle seed flour (3.39 mg GAE/g)) also total flavonoid for turmeric (4.9mg) while and in milk thistle seed flour (2.34mg). DPPH for milk thistle recorded the highest percent of (FRSA) 98.5 % and turmeric recorded lowest present 82.2% in concentration 100 µg/ml. Reducing power for milk thistle recorded 0.651 but turmeric (T) recorded 0.502 in concentration 100 µg/ml . The identification of phenolic compounds for plant extracts were investigated by HPLC ,the highest content in milk thistle seed flour (MTSF) was Benzoic acid (103.33 ppm) while the lowest content was Caffeine(1.07 ppm) but in turmeric(T) the highest content was Benzoic acid (208.41 ppm), while the lowest content was quercetin (3.08 ppm).The level of plasma aspartate aminotransferase (AST) , alanine aminotransferase (ALT) , alkaline phosphatase (ALP) , total bilirubin , total protein and albumin and plasma antioxidant state SOD , CAT, GPx and MDA were determined to assay hepatotoxicity.

**Key words:** Milk thistle seed flour, disorder, Biochemical analyss

### INTRODUCTION

Milk thistle (*Silybum marianum* L. Gaertn) is one of the most ancient known herbal medicines, it is an annual or biennial plant belongs to the family Asteraceae (Compositae). The plant is native to Mediterranean area and now has cultivated in other warm and dry regions (Li et al., 2012). Now *Silybum marianum* is one of the most medicinal plants. The plant has many common names such as, bull thistle, heal thistle, holy thistle, lady's thistle, pig leaves, royal thistle, snake milk, so thistle, St. Mary's thistle, Venus thistle, Marian thistle, Mary thistle, mild thistle, milk ipecac, our lady's thistle (Corchete, 2008). Milk thistle seed contained 20-30% oil, 25-30% protein, 0.038%

tocopherol and 0.63% sterols. Wichtl and Bisset (1994).

Abu Jadayil *et al.*, (1999) reported that milk thistle contained 5.8, 19.1, 26.3, 25.4, 4.8, and 24.3 % for moisture, protein, crude fat, crude fiber, ash and nitrogen-free extract, respectively. Silymarin extracted from milk thistle seed consists of 70-80% flavonolignans and 20-30% polyphenolic compounds. It was reported that the world demand of silymarin is about 18-20 tons per year (Ram *et al.*, 2005). Milk thistle seed involved 0.48% total phenols based on seed dry weight. Khalil (2008) Curcuma naturally found in India to Thailand, Indochina, Malaysia, Indonesia, and finally spreads to northern Australia. Curcuma is extensively cultivated in tropical and subtropical regions of Asia, Australia, Western Africa and South America (Ravindran *et al.*, 2007). The nutritional

compositions of the rhizomes are crude protein (19.44%), lipid (2.5%), and carbohydrate (97.5%). The rhizomes also have a moisture content of 19% and an ash content of 3.21% (Jain and Parihar, (2017). Curcumin (diferuloylmethane) is the compound responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%) (Xiang *et al.*, 2018 ).The total phenolic content of the rhizome extracts of *C. aromatica* is reported in the range of  $151.33 \pm 13.9 \mu\text{g}/\text{mg}$  eq to gallic acid (Jain and Parihar, 2017) to  $265 \pm 1.08 \text{ mg}/\text{g}$  of ascorbic acid (Srividya *et al.*, 2012), and the total flavonoids content ranges from  $106.8 \pm 2.76 \mu\text{g}/\text{mg}$  eq to quercetin (Jain and Parihar, 2017) to  $175 \pm 1.56 \text{ mg}/\text{g}$  of rutin (Srividya *et al.*, 2012). Curcuma phaeocaulis rhizome has 8,9-dehydro-9-formyl- cycloisolongifolene (15.6-46.2%), germacrone (8.9- 21.2%), and curlone (0.8-20.2%) as the main constituents (Zhang *et al.*, 2017).Curcumin increases the intestinal lipase, sucrase, and maltase activity (Su *et al.*, 2017). Curcumin also suppresses the intestinal fibrosis (Lin *et al.*, 2006). Moreover, it has been reported that curcumin has significant effect on dyspepsia and gastric Ulcer and a study showed defensive effects of male Sprague–Dawley (pylorus-ligated) rats treated with curcumin (Kim *et al.*, 2005).

## MATERIALS AND METHODS

### Family name and different names (English and Scientific)

Milk thistle seed flour and turmeric roots samples were collected from local markets and were identified by Plant Department, Faculty Agriculture, Menoufia

English name	Scientific name	Family
Milk thistle	<i>Silybum marianum</i> <i>L. Gaernt</i>	Asterace
Turmeric	<i>Curcuma aromatica</i> , <i>rhizomes</i>	Zingiberaceae

### Chemical composition :

Determination of moisture content: according to ( A.O.A.C. 2000 ).

Determination of crude protein : Total nitrogen was determined ( dry basis ) according to the modified micro-kjeldahl Pirjo and Pekka (1996).

Determination of ash: according to (A.O.A.C. 2000).

Determination of crude fiber: Crude fiber was determined according to (A.O.A.C.2000).

Determination of crude lipid: according to A.O.A.C. (2000).

Determination of total carbohydrate. Total carbohydrate was determined using the following equation Difference =  $100 - (\text{Ash \%} + \text{Protein \%} + \text{Fat \%} + \text{Fiber \%})$

Determination of free phenolic compounds. The concentration of free phenolic compounds in methanol extract was determined colorimetrically by the method of Folin as described by (ulcin, *et al.*, 2002 )

Determination of total flavonoid compounds. The total flavonoid content were determined using the method reported by Dewanto *et al.*, (2002).

### Quantitative Determination of phenolic compounds by HPLC .

Phenolic compounds of *Silybum marianum* or *Turmeric* samples were extracted according to the method describe by Duke *et al.* , (2003). in which a known weight of dried samples was extracted by methanol. Each of phenolic compounds for the two extracts were identified and performed on JASCO HPLC using hypersil C<sup>o</sup> -18 reversed phase column ( 250 x 4.6 ) with 5  $\mu$  particle size. Injection by means of Rhodyne injection valve with 50 PJ fixed loop was used . A constant flow rate of one ml /min was used with two mobile phases solvent ( A ) 0.5 % acetic acid in distilled water at PH 2.65; solvent (B) 0.5% acetic acid in pure ( 99.5 %) acetonitrile , the elution gradient was

linear starting with ( A ) and ending with ( B ) over 35 min using UV detector set at wavelength 254 nm. Phenolic compounds of the samples were identified by comparing their retention times with those of standard mixture. The concentration of an individual compound was calculated on the basis of peak area measurements and then converted to mg/100g dry weight.

#### **Determination of reducing power :**

A spectrophotometric method of (Oyaizu 1986) was used for the measurement of reducing power . For this determination 2.5 ml of each extracts (25µg/ml -50 µg/ml -75 µg/ml -100 µg/ml) were mixed with 2.5 ml of 200 mmol/L sodium phosphate buffer ( PH6.6) and 2.5 ml of 1% potassium ferricyanide . The mixture was incubated at 50C° for 20 min. After adding 2.5 ml of trichloroacetic acid (w/v), the mixture was centrifuged at 650 rpm for 10 min. The upper layer ( 5 ml) was mixed with 5 ml deionized water and 1 ml of 1% of ferric chloride, and the absorbance was then measured at 700 nm. Higher absorbance indicates higher reducing power, where vitamin C was used as standard .

#### **Determination of free radical scavenging activity by DPPH**

Effect of different extracts on DPPH (2, 2 diphenyl-1-picrylhydrazyl ) free radical was measured according to ( Lee *et al.*, 1996). Positive control (standard) was prepared by mixing 4.0 ml of ascorbic acid (0.05 mg/ml) and 1.0 ml of DPPH (0.4g/ml) for aqueous extract, and negative control as a blank, was prepared by mixing extract base ( water and methanol ) with 1.0 ml of DPPH. Four different concentration of extract were mixed with 4.0 ml DPPH , the volume made up to known volume , mixed well and left to stand at room temperature in a dark

place for 30 min. Absorbance was read using a spectrophotometer at 520m. The ability of extract to scavenge DPPH was calculated using the following equation :  
Radical scavenging activity% = (Blank OD-Sample OD)/(Blank OD)×100

#### **Experimental design**

The experimental animals were divided into 4 groups , each having 6 rats as follows ( for 45 days ).The first group was used as normal (negative control) and received tap water as drinking water . The other three groups , received tap water and ethanol (40%v/v) at a dose of (3.76 g/kg body weight) by stomach tube , daily for 45 days, from which the second group (positive control) doesn't have any other treatment drink only ethanol (40%v/v) at a dose of (3.76 g/kg body weight). The third group (ethanol+ aqueous extract of milk thistle seed flour (MTSF) or at dose of 200 mg/kg b.w).The fourth group (ethanol + aqueous extract of *Turmeric* at dose of 200 mg/kg b.w.

#### **Biochemical analysis :**

##### **Liver function tests:**

**Determination of alanine transaminase (ALT) activity:** according to the method of Reitman and Frankel (1957).

**Determination of aspartate transferase (AST) activity:** according to the method of Reitman and Frankel (1957).

**Determination of Bilirubin (Total) :** Bilirubin was determined in plasma as described by ( Tietz,1990)

**Determination of total protein:** Total protein was determined in plasma as described by Schultze and Heremans, (1966) .

**Determination of globulin and A/G Ratio**

Globulin and A/G ratio were calculated according to the formula of (Dumas *et al.*,1971). A/G Ratio was calculated according to the formula A/G

$$\text{Ratio} = \frac{\text{Albumin}}{\text{Globulin}}$$

#### Antioxidant biomarker in vivo :

Determination of Superoxide dismutases (SODs). Superoxide dismutases (SODs) activity was determined in plasma as described by (Nishikimi *et al.*,1972)

Determination of catalase (CAT) activity. Catalase activity was determined in plasma according to (Aebi 1984).

Determination of glutathione peroxidase ( GPX) activity. Glutathione peroxidase (GPx) activity was determined in plasma as described by (Paglia and Valentine , 1967).

Determination of lipid peroxidation (LPO level). Lipid peroxide was determined according to the method (Bulakova *et al.*, 1975).

#### Statistical analyses:

Collected data were subjected to analysis of variance (ANOVA),. Mean's differentiation were compared using Duncan tested at  $p < 0.05$

## RESULTS AND DISSECTION

#### Proximate analysis of milk thistle seed flour and turmeric :

Data in Table (1) showed the proximate analysis of milk thistle and turmeric. It is clear from such data that , our result were in the same line with those found by Anjusha and

Gangaprasad A (2014). Who found that, turmeric is the major species subjected to many studies. It contains protein (6.3%), fat (5.1%), minerals (3.5%) and carbohydrates (69.4%). Also the result were in the same line with those found by Abu Jadayil *et al .*, (1999). Milk thistle seed flour is the major species subjected to many studies. It contains protein (19.1%), fat (26.3%), fiber (25.4%), ash (4.8%), moisture (5.8%) and carbohydrates (18.6%).

#### Total phenolic and total flavonoid of different extracts

Data in Table (2) showed total phenol and total flavonoids methanolic extract of milk thistle seed flour (MTSF) and turmeric (T). It is clear from such data that, the highest mean of turmeric (11.57) while the lowest mean in milk thistle seed flour (3.39) content while total flavonoid, the highest mean of Turmeric (4.9) while the lowest content in milk thistle seed flour (2.34). The above data of turmeric were in the accordance with that obtained by S.W Qader *et al* (2001). In which they mentioned that polyphenol content from 6.15 to 16.07 in ethanolic extract. As for Flavonoid content: Our result of Turmeric were in the accordance with the data obtained by J.C. Tilak *et al* (2004). In which they indicated that it ranging from 3.58 to 7.86 in ethanolic extract. Mean while our result of flavonoid content milk thistle seed flour (MTSF) were in the accordance with that obtained by Bruneton, (1995), who stated that the flavonoids rang from 1.5 to 3%.

Table (1): proximate analysis (w/w%) of milk thistle seed flour and turmeric.

Materials	Parameters (%)					
	Protein	Carbohydrates	Crude lipid	Ash	crude fiber	Moisture
Milk thistle seed flour (MTSF)	13	27.55	15	10.45	26	8
Turmeric(T)	9.3	57	17	6	2.6	8.1

***Hepatoprotective effects of turmeric and milk thistle seed flour against .....***

**Table (2): Total phenol compounds, total flavonoid of milk thistle seed flour and turmeric.**

	<b>Total Phenol (mg GAE/g)</b>	<b>Total flavonoids ( mg catchin /g)</b>
<b>Milk thistle seed flour(MTSF)</b>	<b>3.39</b>	<b>2.34</b>
<b>Turmeric( T )</b>	<b>11.57</b>	<b>4.9</b>

**Free radical scavenging level by DPPH of all extracts of milk thistle seed flour and turmeric**

Data in Fig. (1) showed the result of Free radical scavenging level by DPPH assay % in different concentration 25mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml, milk thistle recorded the higher percent of ( FRSA ) 98.5 % then Turmeric recorded present 82.2% in concentration 100 mg/ml. Our result were in the accordance with the obtained by Khalil,(2008) which DPPH radical scavenging activity of methanol extracts of milk thistle seed flour MTSF was found to be (95.09).

**Reducing power and total antioxidant capacity**

Data in Fig. (2) showed the result of reducing power level assay % in different concentration 25µg/dl, 50 µg/dl, 75 µg/dl, 100µg/dl, milk thistle seed flour (MTSF) and turmeric ( T ) . It is clear from such data that the reducing power of milk thistle seed flour (MTSF) and turmeric increased by increasing the concentration and reach the maxium in milk thistle (0.651) in concentration 100 Mg/dl, while in turmeric (T) reach (0.52) Our result were in the accordance with the obtained by (Shaker *et al.*, 2010). which reported that flavonoids of Milk thistle seed flour (MTSF) had a potent antioxidant effect due to scavenging of free radicals, superoxide anions, and oxygen radical

**HPLC analysis for phenolic compounds on milk thistle seed flour methanolic extract and methanolic extract of turmeric.**

From data given Tables (3 and 4) , it can deduce that Benzoic acid represent the main compound in both milk thistle seed flour (MTSF) and turmeric ( T ) (103.33) ppm and (208.41) ppm respectirely .On the other hand , milk thistle seed flour contain ( 21 ) phenolic compounds wher Turmeric contain (16). Phenolic compounds , analysis of milk thistle seed flour showed that benzoic acid , Myricetin , kampherol , Neringein, Salicylic acid and Ellagic acid are the major phenolic compounds mean while benzoic acid, kampherol, Rosemariric and Myricetin are the major phenolic compounds in Turmeric. The plant phenols , because of their diversity and extensive distribution are considered to be the most important group of natural antioxidants. They posses several common biological and chemical properties , namely antioxidant activity, due to their ability to scavenge active oxygen species or chelate metal ions , as well as their capability to modulate certain cellular enzyme activities (Helser and Hotchkiss 1994).

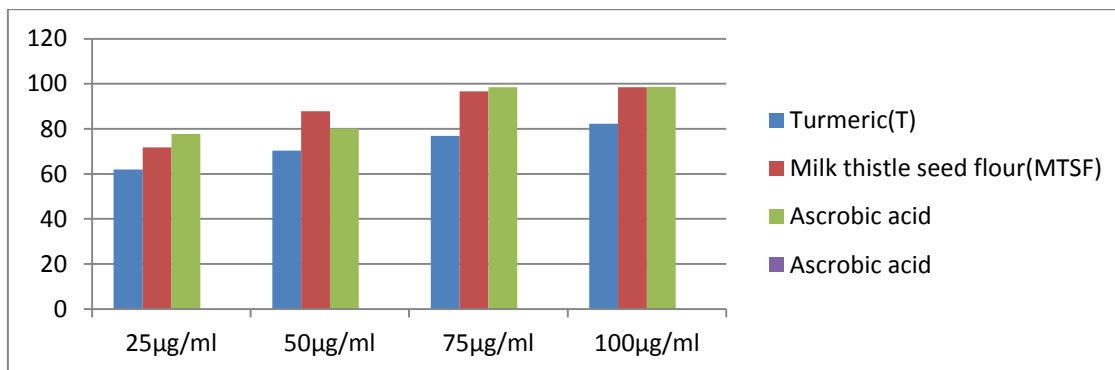
**Effect of all extracts of milk thistle seed flour (MTSF), tumeric on Liver function in rates plasma.**

**Plasma ALT, AST and ALP enzymes**

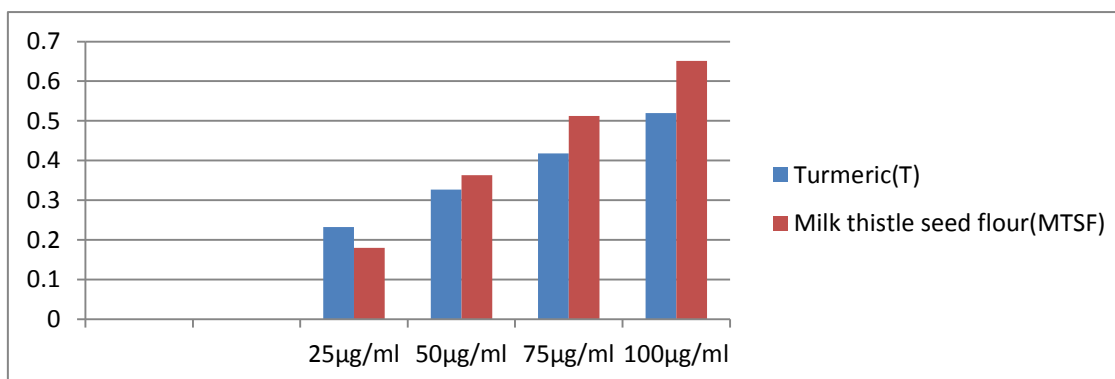
Data in Table (5) showed ( ALT ) level in plasma in all studied groups for 45 days of treatment. It can be noticed that negative control group recorded (37.16 U/L) while the positive control group (which treated with ethanol 40%v/v in drinking water) recorded (84.83 U/L) have the highest values comparing with negative control (37.16 U/L). but the group which treatment with methanol extract milk thistle seed flour (MTSF) at

200mg/kg b.w. decreased to ( 45.83 U/L ).  
In groups which treated with methanol

extract of turmeric at 200mg/kgb.w.  
decreased to ( 55 U/L ).



**Fig. (1): Free radical scavenging level by DPPH of milk thistle seed flour and turmeric**



**Figure (2): Reducing power of milk thistle seed flour and turmeric**

**Table (3): HPLC of milk thistle seed flour.**

Compounds	ppm	Compounds	ppm
Pyrogallol	2.23564	Benzoic acid	103.33696
Quinol	ND	Rutin	5.50555
Gallic acid	9.57633	Ellagic	22.44046
Catechol	ND	O-Coumaric acid	1.77572
P-Hydroxy benzoic acid	ND	Salicylic acid	31.16560
Caffeine	1.07233	Myricetin	95.60416
Chlorogenic	6.96801	Cinnamic acid	1.73047
Vanillic acid	10.63546	Quercetin	2.95785
Caffeic acid	4.12392	Rosemarinic	7.23908
Syringic acid	1.13271	Neringein	31.72992
Vanillin	4.22284	Kampherol	68.65241
P-Coumaric acid	3.10603	Total	394.47703
Ferulic acid	7.51458		

ND= Not detected

***Hepatoprotective effects of turmeric and milk thistle seed flour against .....***

Table (4): HPLC of turmeric.

Compounds	ppm	Compounds	ppm
Pyrogallol	3.02391	Benzoic acid	208.41824
Quinol	4.23620	Rutin	8.29178
Gallic acid	4.46560	Ellagic	13.60961
Catechol	3.01135	O-Coumaric acid	9.34567
P-Hydroxy benzoic acid	ND	Salicylic acid	6.63558
Caffeine	ND	Myricetin	17.99695
Chlorogenic	3.57219	Cinnamic acid	ND
Vanillic acid	ND	Quercitin	3.08968
Caffeic acid	8.49539	Rosemarinic	19.64714
Syringic acid	ND	Neringein	12.23942
Vanillin	ND	Kampherol	94.51375
P-Coumaric acid	ND	Ferulic acid	ND
		Total	388.24070

ND= Not detected

Table (5): effect of milk thistle seed flour and turmeric on ALT and AST and ALP.

Groups	ALT(U/L )	AST(U/L)	ALP(U/L)
	Mean ± S.D	Mean ± S.D	Mean ± S.D
Negative control	37.16±2.86 <sup>d</sup>	108±4.77 <sup>d</sup>	199.16±2.85 <sup>c</sup>
Positive control	84.83±2.32 <sup>a</sup>	145±5.44 <sup>a</sup>	262±8.34 <sup>a</sup>
Milk thistle seed flour(MTSF)	45.83±2.13 <sup>c</sup>	133.83±14.5 <sup>c ±</sup>	198.33±4.55 <sup>c</sup>
Turmeric (T)	55±2.82 <sup>b</sup>	139.67±19 <sup>b</sup>	209.66 ±3.44 <sup>b</sup>

Table ( 5) Values represent mean ± S.D obtained from 6 rats , means in the same column followed by the same letters do not differ significantly , and when the means followed by different letters differ significantly at ( p ≤ 0.05 ).

**Plasma AST enzyme**

Data in Table (5) showed ( AST ) level in plasma in all studied groups for 45 days of treatment. It can be noticed that negative control group recorded (108 U/ml) while positive control group ( which treated with ethanol 40%v/v in drinking water ) recorded (145 U/L have the highest values comparing with negative control (108U/ml). but the group which treatment with methanol extract of milk thistle seed flour (MTSF) at 200mg/kg bw decreased to (133.83 U/L). In groups which methanol extract of turmeric at 200mg /kgb.w. decreased to (139.67 U/L) Our results were in the accordance with the obtained by Shaker *et al.* (2010) which

found that the ethanolic extract of *s. marianum* significantly decreased the elevated liver enzymes caused by CCL<sub>4</sub>, and in the sam line with khajhdehp, *et al*, (2012).

**Alkaline phosphatase (ALP) activity.**

Data in Table (5) showed alkaline phosphatase level in plasma for all studied groups after 45 days of treatment. It can be noticed that negative control group recorded (199.16 U/L) while, positive control group (which treated with ethanol 40%v/v in drinking water) recorded (262 U/ml) have the highest values comparing with negative control (199.1U/L). The group which treatment with

methanol extract milk thistle seed flour 200mg/kg b.w. decreased to (198.33U/L). In groups which methanol extract of turmeric 200 mg/kgb.w. decreased to (209.66U/L). The present result are going in the same line with khajhdehp, et al., (2012).

### Plasma albumin level

Data in Table (6) showed total protein level in plasma for all studied groups after 45 days of treatment. It can be noticed that negative control group recorded (3.88 mg/dl) while, positive control group (which treated with ethanol 40%v/v in drinking water) recorded (3.83 mg/dl). The group which treatment with methanol extract of milk thistle seed flour at 200/kg b.w. increased to (3.97 mg/dl) . In group which treated with methanol of extract turmeric at 200mg/kgb.w. decreased to (3.85 mg/dl) compared with negative control.

### Total protein level

Data in Table (6) showed total protein level in plasma for all studied groups after 45 days of treatment. It can be noticed that negative control group recorded (6.8 mg/dl) while, positive control group (which treated with ethanol 40%v/v in drinking water) recorded (6.65 mg/dl), have the lowest value comparing with negative control (6.8mg/dl). but the group which treatment with methanol extract of milk thistle seed flour at 200mg/kg b.w. increased to (7.28 mg/dl). In groups which methanol extract of turmeric 200 mg/kgbw increased to ( 7.07 mg/dl ).

### Total bilirubin

Data in Table (6) showed total bilirubin level in plasma for all studied groups in 45 days of treatment. It can be noticed that negative control group recorded (0.253 mg/dl) while, positive control group( which treated with ethanol 40%v/v in drinking water ) recorded (0.275 mg/dl ) have the highest values comparing with negative control (0.253mg/dl). but the group which treatment with methanol extract of milk thistle seed flour at 200mg/kg b.w. decreased to (0.235U/ML) . In group which treated with methanol extract of turmeric 200 mg/kgb.w. decreased to (0.198mg/dl). The present result are going in the same line with Suja, et al , (2004) and khajhdehp, et al , (2012) .

### Antioxidant parameters

#### Super oxide dismutases ( SODs)

Data in Table (7) showed SOD level activity in plasma for all studied groups in 45 days of treatment. It can be noticed that negative control group recorded (79.16 U/L) while, positive control group (which treated with ethanol 40%v/v in drinking water) recorded (124.66 U/L) have the highest values comparing with negative control (79.16 U/L). The group which treatment methanol extract of milk thistle seed flour at 200mg/kg b.w. decreased to (117.5 U/L) . In group which treated with methanol extract of turmeric at 200mg/kgb.w. decreased to (112.5 U/L).

Table (6): Effect milk thistel seed and turmeric on albumin, t.protein, globulin and A/G .

Groups	Albumin	T.protein (mg/dl)	T. bilirubin (mg/dl)
	Mean±SD	Mean±SD	Mean±SD
Negative control	3.88±0.1 <sup>a</sup>	6.8±0.26 <sup>a</sup>	0.253±0.07 <sup>a</sup>
Positive control	3.83±0.33 <sup>a</sup>	6.56±0.63 <sup>b</sup>	0.275 ±0.10 <sup>a</sup>
Milk thistle seed flour	3.97±0.19 <sup>a</sup>	7.28±0.45 <sup>a</sup>	0.235±0.04 <sup>a</sup>
Turmeric (T)	3.85±0.23 <sup>a</sup>	7.07±0.6 <sup>a</sup>	0.198 ±0.04 <sup>a</sup>

Table ( 6 ) Values represent mean ± S.D obtained from 6 rats , means in the same column followed by the same letters do not differ significantly , and when the means followed by different letters differ significantly at ( p ≤ 0.05 ).



***Hepatoprotective effects of turmeric and milk thistle seed flour against .....***

**Table (7): Effect of milk thistle seed flour and turmeric on SOD , CAT , GPx ,MDA.**

Groups	SOD( U/L)	CAT( U/L)	GPX( U/L)	MDA( U/L)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Negative control	79.16±5.23 <sup>d</sup>	27.33±3.3 <sup>d</sup>	41.33±2.42 <sup>c</sup>	4.28±0.4 <sup>c</sup>
Positive control	124.66±8.6 <sup>a</sup>	48.5±1.87 <sup>a</sup>	62.33±3.88 <sup>a</sup>	6.96±0.69 <sup>a</sup>
Milk thistle seed flour	117.5±2.17 <sup>b</sup>	44± 2.82 <sup>b</sup>	50.16±4.2 <sup>b</sup>	6±0.22 <sup>b</sup>
Turmeric (T)	112.5±52.3 <sup>c</sup>	37.33±2.16 <sup>c</sup>	48.33±4.5 <sup>b</sup>	5.59±0.28 <sup>b</sup>

Table (7) Values represent mean ± S.D obtained from 6 rats , means in the same column followed by the same letters do not differ significantly , and when the means followed by different letters differ significantly at (  $p \leq 0.05$  ).

**Catalase level**

Data in Table (7) showed Catalase level in plasma for all studied groups after 45 days of treatment. It can be noticed that negative control group recorded (27.33 U/L) while, positive control group (which treated with ethanol 40%v/v in drinking water) recorded (48.5 U/L) have the highest values comparing with negative control (27.33 mg/dl). but the group which treatment methanol extract milk thistle seed flour at 200mg/kg b.w. decreased to (44.0 U/L). In group which treated with methanol extract of turmeric 200 mg/kgb.w. decreased to (37.33 U/L).

**Glutathione peroxidase (GPx) activity**

Data in Table (7) showed GPx activity level in plasma for all studied groups after 45 days of treatment. It can be noticed that negative control group recorded (41.33 U/L) while, positive control group (which treated with ethanol 40%v/v in drinking water ) recorded(62.33 U/L) have the highest values comparing with negative control ( 41.33) U/L. but the group which treatment with methanol extract of milk thistle seed flour200 at mg/kg b.w. decreased to( 50.16 U/L) . In group which teated with methanol extract of turmeric at 200mg/kgb.w. decreased to (48.33 mg/dl ).

**Malondialdehyde level :**

Data in Table (7) showed MDA level in plasma for all studied groups after 45 days of treatment. It can be noticed that negative control group recorded (4.28 U/L) while, positive control group (which treated with ethanol 40%v/v in drinking water) recorded (6.96 U/L) have the highest values comparing with negative control (4.28 U/L). but the group which treatment with methanol extract milk thistle seed flour at 200 mg/kg b.w. decreased to (6.0 U/L) . In group which treaded with methanol extract of turmeric 200 mg/kgb.w. decreased to (5.59 U/L). The result are in the same line with Toklu *et al* (2008) they stated that MDA recorded in negative control 0.96 and positive control recorded 6.64 mean whil methanol extract milk thistle seed flour recorded 5.01 due to the antioxidant properties of flavonoids which present in the plant.

**REFERENCES**

A.O.A.C Association of Official Analytical chemists (2000). Official Methods of Analytical Chemistry, Washington, D.C, MSA.  
 Abu Jadayil, S., S. K. Tukan and H. R. Takruri (1999). Bioavailability of iron from four different local food plants in Jordan. Plant Foods for Human Nutrition, 54: 285- 294.

- Aebi, H. (1984). Catalase assay. Method Enzymol. 150: 121-126
- Anjusha, S. and A. Gangaprasad (2014). Phytochemical and Antibacterial Analysis of Two Important Curcuma Species, *Curcuma aromatica*, *Salisb. and Curcuma /anthorrhiza Roxb.* (Zingiberaceae). Journal of Pharmacognosy and Phytochemistry 3(3): 50-53
- Bruneton, J. (1995). Pharmacognosy, phytochemistry, medicinal plants. Lavoisier publishing.
- Bulakova, E.B., A.V. Alesenko, E.M. Molochkina, N.P. Plasma, D.C. Cannon, I. Olitzky and J.A. Inkpen (1975). Proteins . In; Clinical chemistry principles and technics. 2-nd ed RJ Henery . DC Cannon, JW Winkelman, editors, Harper and Wow, New York, pp 407-421.
- Corchete, P. (2008). *Silybum marianum* (L.) Gaertn: the source of silymarin. In bioactive molecules and medicinal plants. Springer Berlin Heidelberg, 123- 148.
- Dewanto, V., X. Wu, K.K. Adom and R.H. Liu (2002). Thermal processing enhances the nutritonalvalue of tomatoes by increasing total antioxidant activity .J Aric Food Chem 50: 3010-3014.
- Doumas, B.T., W.A. Watson and H.G Biggs (1971). Albumin standards and the measurement of serum albumin with bromocresol green Clinica Chimica Acta , 31: 87-93.
- Duke, S.O., A.M. Rimando, P.F. Pace, K.N. Reddy and R.J. Smenda (2003). Isoflavon, glyphosate and aminoethylphosphonic acid levels in seeds of glyphosate - treated, glyphosate- resistant soybean. J. Agric. Food Chem., 5 (1): 340-344.
- Forsyth, JE, S. Nurunnahar, S.S. Islam, M. Baker, D. Yeasmin, M.S. Islam, M. Rahman, S. Fendorf, N.M. Ardoin, P.J. Winch and S.P. Luby (2019). Turmeric means “yellow” in Bengali: Lead chromate pigments added to turmeric threaten public health across Bangladesh. Environmental research. 2019 Dec 1; 179: 708-722.
- Gulcin, I., M. Oktay, I. Kufrevioglu and A. AsIn (2002). Determination of a ntioxidant activity of lichen *Cetrariaislandica* (L.) Ethnopharmacol. 79: 325-29.
- Han, L., Y. Zheng, B. Xu, H. Okuda and Y. Kimura (2002). Saponins from *Platycodi radix* ameliorate high fat diet- induced obesity in mice. American Society Nutrition Science J.; 132: 2241-2245.
- Helser, M.A. and J.H. Hotchkiss (1994). Comparison of tomato phenolic and ascorbate fractions on the inhibition of N-nitroso compound formation. journal of agricultural and food chemistry. 42:129 – 132 .
- Ismael, S. M., A. M. Farahat, Y. M. Ebrahim and S. T. Gohari (2014). Functional and nutritional properties of stirred yoghurt supplemented with silymarin and its impact on chronic hepatic damage. World J. Dairy & Food Sci.9 (1): 36-50.
- Tilak, J. C. M., H. Banerjee and T. P. A. Mohan (2004). Devasagayam, “Antioxidant availability of turmeric in relation to its medicinal and culinary uses,” *Phytotherapy Research*, vol. 18, no. 10, pp. 798–804, View at: [Publisher Site](#) | [Google Scholar](#)
- Jain, A. and D.K. Parihar (2017). Nutritional evaluation of *Curcuma* species collected from different agro climatic regions of chhattisgarh. Am J Ethnomed, 04:1–8; doi:10.21767/2348-9502.100020
- Khajehdehi, P. (2012). Turmeric: Reemerging of a neglected Asian traditional remedy. Journal of nephropathology. 1(1): 17.
- Khalil, A. J. (2008). Biochemical studies on milk thistle (*Silybummarianum*). M.Sc.; Thesis, Fac. Agric., Cairo univ.; Egypt
- Kim, D.C., S.H. Kim, B.H. Choi, N.I. Baek, Daeho Kim, M.J. Kim and K.T. Kim (2005). *Curcuma longa* extract protects against gastric ulcers by

- blocking H2 histamine receptors. *Biological and Pharmaceutical Bulletin* 28: 2220-2224
- Koulshon, A., G. Spiller and J. W. Farquhar (2005). Effect of a plant based diet on plasma lipids in hypercholesterolaemic adults. *Annual Int. Medicines*, 142: 725 - 755.
- Lee J., J. Park and Choi (1996). The antioxidant activity of *Ecklonia stolonifera*. *Archives Pharmacol Res.*, 19(3): 223-227.
- Li, F., L. Yang, T. Zhao, J. Zhao, Y. Zou, Y. Zou and X. Wu (2012). Optimization of enzymatic pretreatment for n-hexane extraction of oil from *Silybum marianum* seeds using response surface methodology. *Food and bioproducts processing*, 90(2): 87- 94.
- Lin, X., L. Xue, H. Zhang and C. Zhu (2006). Determination of curcumins in turmeric by micellarelectro kinetic capillary chromatography. *Canadian Journal of Analytical Sciences and Spectroscopy* 51:35-42.
- Nishikimi, M., N.A. Roa and K. Yogi (1972). Measurement of superoxide dismutase. *Biochem. Biophys Res. Common*, 46: 849-854
- Oboh, H. A. and C. O. Omofoma (2008). The effects of heat treated lima bean (*Phaseolus lunatus*) on plasma lipids in hypercholesterolaemic rats. *Pakistan J. of Nutrition* 7: 636- 639.
- Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *J Nutr*. 7:307-315.
- Ozturk, M., M. Akdogan, I. Keskin, A. N. Kisioglu, S. Oztas and K. Yildiz (2012). Effect of *Silybum marianum* on acute hepatic damage caused by carbon tetrachloride in rats. *Biomed Research*, 23(2): 268- 274.
- Paglia, D.E. and W.N. Valentine (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J.Lab. Clin.Med.*70:158-169.
- Pirjo, P.S. and E.K. Pekka (1996). Determination of protein in foods: comparison of net protein and crude protein (NX6.25) values. *Food chemistry* .57(1):27-31.
- Ram, G., M. K. Bhan, K. K. Gupta, B. Thaker, U. Jamwal and S. Pal (2005). Variability pattern and correlation studies in *Silybum marianum* Gaertn.
- Ravindran, N., K. Nirmal and K. Sivaraman (2007). *Tumeric the genus Curcuma*. CRC Press, London, UK,.
- Reitman, S. and S. Frankel (1957). A colorimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J.Clin. Path.*, 28: 57-63.
- Qader, S. W., M. A. Abdulla, L. S. Chua, N. Najim, M. M. Zain and S. Hamdan (2001). "Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants," *Molecules* 16: 3433-3443.
- Schultze, H.E. and J.F. Heremans (1966). *Molecular biology of human protein*. Elsevier publishing company, Amsterdam. 1; section 3, chap3.
- Shaker, E., H. Mahmoud and S. Mnaa (2010). Silymarin the antioxidant component and *Silybummarianum* prevents liver damage. *Food Chem. Toxicol.*, 48 (3): 803-806
- Siewek Siewek F. *Exotische Gewürze Herkunft Verwendung Inhaltsstoffe* (in German). Springer-Verlag. 2013 : 72. ISBN 978-3-0348-5239-5. *Medicine* 92: 57-66.
- Singh, R. B., G. Dubnor, M. A. Niaz, S. Gosh, R. Singh, S. S. Rastogi, O. Manor, D. Pella and E. M. Berry (2002). Effect of an indo-Mediterranean diet on progression of coronary heart disease in high risk patients. *Lancet*, 360: 1344-1355.
- Skottová, N., R. Večeřa, K. Urbánek, P. Vána, D. Walterová and L. Cvak (2003). Effects of polyphenolic fraction of silymarin on lipoprotein profile in rats fed cholesterolrich diets. *Pharmacol. Res.*, 47: 17-26
- Srividya, A.R., P. Dhanabal, P. Bavadia, V.J. Vishnuvarthan, Sathish and MN.

- Kumar (2012). Antioxidant and antidiabetic activity of *Curcuma aromatica*. Int J Res Ayurveda Pharm, 3: 401–405.
- Su, X., B. Jiang, H. Wang, C. Shen, H. Chen and Li. Zeng (2017). Curcumin suppresses intestinal fibrosis by inhibition of PPAR $\gamma$ -mediated epithelial-mesenchymal transition. Evidence-Based Complementary and Alternative Medicine 92: 57-66.
- Suja, S.R., P. G. Latha, P. Pushpangadan and S. Rajasekharan (2004). Evaluation of hepatoprotective effects of *Helminthostachys zeylanica* L. hook against carbon tetrachloride-induced liver damage in Wistar rats. J. Ethnopharmacol. 92: 61-66.
- Therhault, A., Q. Wang, S. C. Van Iderstine, B. Chen, A. A. Franke and K. Adeli (2000) . Modulation of hepatic lipoprotein synthesis and secretion by taxifolin, a plant flavonoid. J. Lipid Res. 41: 1969-1979.
- Tietz NW, ed (1990). Clinical Guide to laboratory testes. 2 nd ed. Philadelphia: WB Saunders : 26 – 29 .
- Toklu, H.Z., T.T. Akbay, A. Velioglu-Ogunc, F. Ercan, N. Gedik and M. Keyer-Uysal (2008). Silymarin, the antioxidant component of *Silybum marianum*, prevents sepsis-induced acute lung and brain injury. Journal of Surgical Research. 145(2): 214-222.
- Wahid, A., A. N. Hamed, H. M. Eltahir and M. M. Abouzied (2016). Hepatoprotective activity of ethanolic extract of *Salix subserrata* against CCl<sub>4</sub>-induced chronic hepatotoxicity in rats. BMC Complementary & Altern. Med. 16: 263-273.
- Wichtl, M. and N. G. Bisset (1994). Herbal Drugs and Phytopharmaceuticals Stuttgart: Medpharm Scientific Publishes.2016.Hepatoprotective activity of ethanolic extract of *Salix subserrata* against CCl<sub>4</sub>-induced chronic hepatotoxicity in rats. BMC Complementary & Altern. Med. 16:263-273.
- Xiang, H., L. Zhang, Xi. Lu, Y. Yang, X. Wang, D. Lei, Xi Zheng and X. Liu (2018). Phytochemical profiles and bioactivities of essential oils extracted from seven *Curcuma* herbs, Industrial crops and products 111:298-305
- Zhang, L., Z. Yang, J. Wei, P. Su, W. Pan, X. Zheng, K. Zhang, L. Lin, J. Tang, Fang and Z. Du (2017). Essential oil composition and bioactivity variation in wild-growing populations of *Curcuma phaeocaulis* Valet collected from China. Industrial Crops and Products 103: 274-282

## دراسة تأثير بذور شوك الجمل وجذور الكركم على السمية الكبدية التي يسببها الإيثانول في الفئران البيضاء

فؤاد مطاوع الشوني، سمير عبد القادر القدوسي، صلاح منصور عبد الجواد،

خالد عبد الرحيم الشحات الخلوي

قسم الكيمياء الحيوية الزراعية - كلية الزراعة - جامعة المنوفية.

### الملخص العربي

يما يلي ملخص للنتائج التي تم الحصول عليها:

التركيب الكيميائي الإجمالي للنباتات المختبره: محتويات دقيق بذور شوكة الجمل ، البروتين (13.0%) ، الكربوهيدرات (27.55%) ، الدهن الخام (15.0%) ، الرماد (10.45%) ، الألياف الخام (26.0%) ، الرطوبة (8.0%) لكن بروتين الكركم (9.3%) ، كربوهيدرات (57%) ، دهون خام (17.0%) ، رماد (6.0%) ، ألياف خام (2.6%) ، رطوبة (8.1%) إجمالي الفينولات الكلية يمكننا أن نستنتج أن أعلى متوسط للكركم (11.57 مجم GAE / جم) بينما أدنى متوسط في دقيق بذور شوكة الجمل (3.39 مجم GAE / جم). مجموع الفلافونويد يمكننا أن نستنتج أن ، أعلى متوسط للكركم (4.9 مجم GAE / جم) بينما أدنى متوسط في دقيق بذور شوكة الجمل (2.34 مجم GAE / جم). في حين أن فحص DPPH الجذري بتركيزات مختلفة 25 مجم / ديسيلتر ، 50 مجم / ديسيلتر ، 75 مجم / ديسيلتر ، 100 مجم / ديسيلتر من شوكة الجمل سجل أعلى نسبة (98.5) %FRSA وسجل الكركم أقل نسبة 82.2% في التركيز 100. نتيجة فحص مستوى القدرة بتركيزات مختلفة 25 مجم / ديسيلتر ، 50 مجم / ديسيلتر ، 75 مجم / ديسيلتر ، 100 مجم / ديسيلتر ، سجل دقيق بذور شوكة الجمل (MTSF) أعلى نسبة (انخفاض مستوى الطاقة) 0.651% لكن الكركم سجلت (T) أقل 0.502% في التركيز 100%. يمكن أن نستنتج أن أعلى محتوى لدقيق بذور شوكة الجمل (MTSF) كان حمض البنزويك (103.336) جزء في المليون بينما أقل محتوى كان الكافيين (1.072) ولكن في الكركم (T) أعلى محتوى كان حمض البنزويك (208.41) جزء في المليون تمت دراسة تأثير بذور شوكة الجمل وريزومات الكركم على السمية الكبدية المستحثة في الفئران البيضاء بالجرعة الحادة من الإيثانول ، وقسمت الحيوانات إلى 4 مجموعات: قسمت حيوانات التجربة إلى 4 مجموعات ، كل منها 6 فئران على النحو التالي (لمدة 45 يوماً) .تم استخدام المجموعة الأولى كالمعتاد (المجموعة السلبية) وحصلت على ماء للشرب. أما المجموعات الأربع الأخرى فقد تلقت المياه والإيثانول (40% حجم / حجم) بجرعة (3.76 مل / كجم من وزن الجسم) عن طريق أنبوب المعدة ، يومياً لمدة 45 يوماً ، ولم يتم الحصول على المجموعة الثانية (المجموعة الإيجابية). تناول فقط الإيثانول (40% حجم / حجم) بجرعة (3.76 مل / كجم من وزن الجسم). المجموعة الثالثة (إيثانول + مستخلص مائي من دقيق بذور شوكة الجمل (MTSF) بجرعة 200 ملجم / كجم من وزن الجسم) المجموعة الرابعة (إيثانول + مستخلص مائي من الكركم بجرعة 200 ملجم / كجم من وزن الجسم) تم قتل الفئران ودماء. تم جمع العينات من تقنية أورد الجيوب الأنفية المدارية باستخدام أنابيب الشعر الهيبارين في نهاية الفترة ويمكن تلخيص النتائج التي تم الحصول عليها على النحو التالي:أوضحت النتائج الحالية أن الإيثانول تسبب في ارتفاع مستويات ALT و AST و ALP والبليروبين الكلي والجلوبيولين في البلازما. كما تسبب في انخفاض معنوي ( $P > 0.05$ ) في البروتين الكلي ، الألبومين . أدت التغذية على بذور شوكة الجمل أو الكركم إلى تقليل ALT و AST و ALP و البليروبين الكلي. أيضا زيادة في تركيز البروتين الكلي والألبومين. قد يكون هذا التأثير بسبب نشاطاته القوية المضادة للأكسدة والمضادة للالتهابات لكل من بذور شوكة الجمل والكركم. أوضحت النتائج الحالية أن الإيثانول تسبب في ارتفاع نسبة الجلوكوز في البلازما. أدت التغذية على بذور شوكة الجمل أو الكركم إلى انخفاض نسبة الجلوكوز. وضحت النتائج الحالية أن الإيثانول تسبب في ارتفاع كل ما يلي في البلازما SOD و CAT و GPx و MDA. كما تسبب في انخفاض كبير في كل ما يلي ( $p > 0.05$ ) في SOD و CAT و GPx و MDA

التأثيرات الواقية للكبد من الكركم ومسحوق بذور شوكة الجمل ضد تلف الكبد الناتج عن الإيثانول في الجرذان الوليدية

أسماء السادة المحكمين

أ.د/ عماد محمد عبدالحليم الخلوي كلية الأقتصاد المنزلي - جامعة المنوفية

أ.د/ مصطفى عبد الله همام كلية الزراعة - جامعة المنوفية