

Effect of Doum And Methionine Combination on Hepatotoxicity in Rats

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Abstract: The present study was performed to evaluate the effect of doum and methionine on nutritional status and liver functions in cirrhotic rats by carbon tetrachloride. Forty eight white male albino rats (Sprague dawley strain) were injected by CCl₄ to induce hepatotoxicity. Rats were randomly classified into control (+ve), and 5 treated groups which were drug, doum powder, doum extract, doum powder with methionine and doum extract with methionine groups. Results clearly revealed that consumption of doum and methionine alone showed improvement of healthy status. The best treatments were consumption of doum powder or doum extract with methionine which had the best nutritional results as increasing of weight gain, food efficiency ratio and protein efficiency ratio and had lowest values of Serum cholesterol, total lipid, and some liver function enzymes (alanine and aspartate aminotransferase, alkaline phosphatase and gamma glutamyle transferase). Also, they showed highest values of glycogen, triglyceride, hemoglobin, packed cell volume, and some liver antioxidant enzymes (superoxide dismutase, glutathione-peroxidase, glutathione S-transferase and catalase) compared to control(+ve) group. It could be concluded that consumption of doum and methionine has a best significant treatment effect against hepatotoxicity induced by CCl₄ in rats.

Key words: Doum-methionine – CCl₄ – hepatotoxicity – rats.

INTRODUCTION

The liver is the largest most complex organ in the body and plays major roles in a wide variety of biochemical functions of the body. Therefore, diseases of the liver will markedly affect health. Liver dysfunction can therefore significantly impact many other organs and systems throughout the body (Finkelstein 1990). However, herbal medicines are known to play an important role in the treatment of hepatopathy (Mitra and Sundaram 2007).

L-Methionine is one of eight essential amino acids, which means that it is an essential building block for proteins and it cannot be produced in the body so, ingestion in diet and supplements is required (Bianchi *et al.*, 2000). L-Methionine is beneficial for treating liver disease, and is recommended for people who adhere to a vegetarian diet. It also benefits skin tone and elasticity, nails and hair (McClain *et al.*, 2002).

The doum palm (*Hyphaene thebaica*) is a tree of the palm family. The fruits of doum is oval, shiny, and red to orange in colour, with an average length and diameter of 6 and 5 cm, respectively. The fruit has a brown outer fibrous flesh. The pericarp and the outer coat of the endocarp are inedible, while the mesocarp and kernel flesh are edible. Doum palm kernel is edible when it is unripe but hard when it is ripe. The Doum may play a role in regulating blood pressure, has a cooling and soothing effect on the digestive system and antioxidant properties which help flush toxins from the body (Hsu *et al.*, 2006 and Orwa *et al.*, 2009).

Therefore, the present study is aimed to evaluate the treated effect of methionine and doum palm fruits power or extract on hepatotoxicity in experimental rats.

MATERIALS AND METHODS

I- Materials:

A total of forty eight white male albino rats (Sprague dawley strain), weighing between (105±5g) provided from experimental animals center in Medicine collage of King Saud University in Riyadh. The basal diet consisted of protein (13%), fat (4%), salt mixture (3.5%), vitamin mixture (1%), choline (0.2%), cellulose (5%) and the remainder was starch according to Reeves *et al.*, (1993). Carbon tetrachloride CCl₄ was obtained from El-Gomheria Co. Silymarin as the reference hepatoprotective drug was obtained from CID Co. (Cairo, Egypt). Methionine was purchased from Sigma Chemical Co., in Riyadh. Dried doum palm (*Hyphaene thebaica*) powder obtained from local market in Riyadh.

II- Methods:

1 – Preparation of Doum:

Dried doum palm (*Hyphaene thebaica*) was crushed to fine powder and added as 10% of the basal diet. To prepare doum extract, 5kg doum powder was extracted by 70% ethanol on cold until exhaustion. The solvent was distilled in rotary evaporator at 55°C till dryness and dissolved in double distilled water for final administration (WHO 1983).

3- Biological Deign:

Rats fed on basal diet for a week as adaptation period in wire cages under the normal laboratory conditions. All rats were subcutaneously injected by carbon tetrachloride CCl₄ that diluted by paraffin oil (1:1) in a dose of 2 ml /kg of body weight of rat, twice in the week during the experimental period to induce hepatotoxicity according to the method described by Wilfried *et al.*, (1994) . The rats were randomly classified into six groups (8 rats each) which were control (+ve), and 5 treated groups which classified as following:

Silymarin group: Administered basal diet and 100 mg/kg silymarin by stomach tube.

Doum powder group: Administered basal diet containing 10% doum powder.

Doum extract group: Administered basal diet and 25 mg /kg body weight daily doum extract by stomach tube.

Doum powder with methionine group: Administered basal diet containing 10% doum powder and 5 g/kg of methionine by stomach tube.

Doum extract with methionine group: Administered basal diet and 25 mg /kg body weight daily doum extract with 5 g/kg of methionine by stomach tube.

Food and water were provided ad-libitum. The experiment continued for sixty day. The food intake was calculated daily and the body weight gain was recorded weekly. Food efficiency ratio (FER) and protein efficiency ratio (PER) were determined by Chapman *et al.*, (1950). At the end of experiment, rats were anesthetized, blood sample were collected from hepatic portal vein in clean centrifuge tubes. Liver was removed and blotted on filter paper. Hemoglobin (HG) and packed cell volume (PCV) were estimated in heparinized blood according to Drabkin (1949) and Mc Inory (1954), respectively. Serum alanine and aspartate aminotransferase (ALT, AST) activity, alkaline phosphatase (ALP) and gamma glutamyle transferase (γ GT) were estimated according to Bergmeyer and Horder (1980), Kind and King (1954), and Henry, (1974), respectively. Liver glycogen, triglyceride, total cholesterol and total lipid were estimated according to Rerup and Lundquist (1967), Scheletter and Nussel (1975), Richmond (1973), Folch *et al.* (1957), respectively. Liver superoxide dismutase (SOD), glutathione-peroxidase (GPX) , glutathione S-transferase (GST), catalase and malondialdehyde (MDA) were estimated according to Misra and Fridovich (1972), Rotruck *et al.*, (1973) , Ellman (1958), Sinha (1972), and Ohkawa *et al.* (1979)., respectively.

Statistical Analysis:

Data are expressed as mean \pm SE. Statistical analysis was done by using analysis of variance (ANOVA) followed by student’s t-test and P values of 5% and less were considered to be significant (Artimage and Berry 1987).

Results:

The effect of doum and methionine consumption on weight gain, food intake, food efficiency ratio (FER) and protein efficiency ratio (PER) were illustrated in table (1). It could be observed that silymarin group had significant increase in body weight gain, FER and PER (P<0.01). Moreover, consumption of doum powder or extract could improve nutritional results that represented by increasing the body weight gain, FER and PER (P<0.01) and that result were more obviously when doum powder or extract consumed with methionine (P<0.001) comparing with control (+ve) group. Also, consumption of doum powder or extract with methionine showed significant increase in nutritional results comparing with silymarin group.

Table 1: Mean values \pm SD of body weight gain, food intake and FER of the experimental rat groups.

Groups Variables	Control (+ve)	Silymarin	Doum powder	Doum extract	Doum powder + methionin	Doum extract + methionin
Weight gain	75.81 \pm 10.36c	118.83 \pm 15.16b**	125.55 \pm 22.11ab*	120.61 \pm 21.17ab**	130.71 \pm 25.14a***	137.31 \pm 31.71a***
Food intake	15.36 \pm 1.78a	17.81 \pm 1.36a	17.67 \pm 1.71a	18.11 \pm 2.11a	17.59 \pm 1.55a	18.24 \pm 1.24a
FER	0.082 \pm 0.003c	0.111 \pm 0.02b**	0.118 \pm 0.01b**	0.110 \pm 0.001b**	0.123 \pm 0.004a***	0.125 \pm 0.005a***
PER	0.41 \pm 0.02c	0.55 \pm 0.03b**	0.59 \pm 0.01ab**	0.55 \pm 0.02b**	0.62 \pm 0.03a***	0.63 \pm 0.04a***

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
 Values with the same letters indicate non- significant difference (P<0.05) and vice versa.

Data in table (2), it could be observed that silymarin group showed a significant decrease in the values of serum ALT, AST, ALP and γ GT at $p < 0.01$ & 0.001 compared with control (+ve) group.

Consumption of doum powder or extract only or with methionine showed a significant decrease in ALT and γ GT at $p < 0.01$ while consumption of doum powder or extract showed a significant decrease in AST ($p < 0.01$) and ALP ($p < 0.001$) but consumption of doum powder or extract with methionine showed a significant decrease in AST ($p < 0.01$ & 0.001) and ALP ($p < 0.001$) compared with control (+ve) group. There were non significant difference in ALT and γ GT among all treated groups. Doum powder or extract groups showed a significant increase in AST and ALP compared with doum extract with methionine group.

Table 2: The Mean values \pm SD of serum ALT, AST, ALP and γ GT of the experimental rat groups.

	Control (+ve)	Silymarin	Doum powder	Doum extract	Doum powder + methionin	Doum extract+ methionin
ALT	148.96 \pm 24.71a	97.96 \pm 11.23b**	108.70 \pm 19.61b**	98.51 \pm 8.91b**	101.31 \pm 16.71b**	95.78 \pm 11.22b**
AST	165.71 \pm 31.60a	110.21 \pm 15.11bc*	119.65 \pm 21.88b**	108.71 \pm 12.12bc**	112.14 \pm 17.31b**	105.40 \pm 18.81c**
ALP	190.67 \pm 33.60a	118.71 \pm 9.67c***	136.70 \pm 18.81b**	128.11 \pm 15.71b**	110.21 \pm 12.31c**	112.78 \pm 10.71c**
γ GT	19.69 \pm 2.11a	13.21 \pm 1.76b**	14.96 \pm 1.47b**	13.91 \pm 2.11b**	12.71 \pm 1.36b**	12.36 \pm 1.71b**

Significant with control (-ve) group * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Values with the same letters indicate non- significant difference ($P < 0.05$) and vice versa.

Data recorded in table (3) showed that silymarin group had significant increased in the value of HG ($p < 0.05$) but doum powder and extract only or with methionine groups showed significant increased in the value of HG ($p < 0.01$ & 0.001) and PCV ($p < 0.05$ & 0.01) compared with control (+ve) group. The value of HG was in non significant difference among doum powder and extract only or with methionine groups but the value of PCV was significantly increased in doum extract, doum powder with methionine and doum extract with methionine groups compared with silymarin group.

Table 3: Mean values \pm SD of HG and PCV of the experimental rat groups.

	Control (+ve)	Silymarin	Doum powder	Doum extract	Doum powder + methionin	Doum extract+ methionin
HG	9.11 \pm 0.88c	11.31 \pm 0.99ab*	12.11 \pm 1.21a**	12.96 \pm 1.03a**	13.11 \pm 1.21a***	13.55 \pm 1.11a***
PCV	25.11 \pm 5.11c	29.66 \pm 6.11bc	32.10 \pm 6.21ab*	35.17 \pm 7.36a**	36.11 \pm 7.11a**	38.70 \pm 7.03a**

Significant with control (-ve) group * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Values with the same letters indicate non- significant difference ($P < 0.05$) and vice versa.

Data presented in table (4) showed that silymarin group showed significant increase in liver glycogen ($p < 0.01$) and significant decrease in liver total lipids ($p < 0.01$) while doum powder group showed significant decrease in liver cholesterol and total lipids ($p < 0.05$ & 0.01) but doum extract group showed significant increase in liver glycogen ($p < 0.01$) and significant decrease in liver cholesterol ($p < 0.05$) and total lipids ($p < 0.01$) compared with control (+ve) group. Doum powder and extract with methionine groups showed significant increase in liver glycogen and triglyceride ($p < 0.001$) and significant decrease in liver cholesterol ($p < 0.05$) and total lipids ($p < 0.001$) compared with control (+ve) group. There were non significant difference in liver glycogen and cholesterol in doum powder and extract only or with methionine groups compared with silymarin group. Doum powder and extract with methionine groups showed significant increase in liver triglyceride and significant decrease in liver total lipids compared with silymarin group.

Table 4: The Mean values \pm SD of liver glycogen, triglyceride, total cholesterol and total lipid of the experimental rat groups.

	Control (+ve)	Silymarin	Doum powder	Doum extract	Doum powder + methionin	Doum extract+ methionin
Glycogen	3.41 \pm 0.88c	5.71 \pm 2.01ab**	4.96 \pm 1.11bc	5.81 \pm 1.01ab**	6.11 \pm 1.55a***	6.56 \pm 1.36a***
Triglyceride	1.42 \pm 0.33bc	1.96 \pm 0.66b	1.69 \pm 0.53b	1.98 \pm 0.47ab	2.11 \pm 0.58a**	2.31 \pm 0.57a**
total cholesterol	7.51 \pm 1.76a	6.11 \pm 1.21ab	4.33 \pm 1.41b*	5.11 \pm 1.22b*	4.51 \pm 1.30b*	4.17 \pm 1.12b*
Total lipid	65.91 \pm 6.77a	55.83 \pm 6.11b**	49.61 \pm 5.14b**	45.71 \pm 5.11bc**	41.31 \pm 4.18c***	40.39 \pm 4.71c***

Significant with control (-ve) group * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Values with the same letters indicate non- significant difference ($P < 0.05$) and vice versa.

Data in table (5) revealed that all treated groups showed a significant increase in the values of liver SOD, GPX, GST and catalase and a significant decrease in MDA at $p < 0.01$ & 0.001 when compared with control (+ve) group. Doum extract, doum powder with methionine and doum extract with methionine groups showed

significant increase in SOD, GPX, GST and catalase and a significant decrease in MDA when compared with silymarin group.

Table 5: The Mean values \pm SD of liver SOD, GPX, GST, catalase and MDA of the experimental groups.

	Control (+ve)	Silymarin	Doum powder	Doum extract	Doum powder + methionin	Doum extract+ methionin
SOD(μ /mg)	22.88 \pm 4.21d	35.11 \pm 4.01c**	45.3 \pm 5.61b***	49.96 \pm 6.60ab***	53.21 \pm 7.11a***	55.11 \pm 6.77a***
GPX(μ /mg)	19.87 \pm 2.19c	29.14 \pm 3.61b**	32.81 \pm 4.59b**	40.11 \pm 5.58a***	42.17 \pm 3.96a***	45.39 \pm 4.77a***
GST(μ /mg)	1.15 \pm 0.11d	2.11 \pm 0.22c**	3.14 \pm 0.56b***	3.51 \pm 0.58b***	3.96 \pm 0.77b***	4.11 \pm 0.88a***
Catalase(μ /mg)	18.11 \pm 1.29c	34.41 \pm 4.25b***	39.61 \pm 3.66ab***	41.39 \pm 3.81a***	45.37 \pm 5.17a***	44.96 \pm 6.01a***
MDA	23.71 \pm 3.23a	13.24 \pm 2.11b**	10.29 \pm 1.72bc***	9.61 \pm 2.11c***	9.17 \pm 1.99c***	9.36 \pm 2.11c***

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Values with the same letters indicate non- significant difference (P<0.05) and vice versa. 95407

Discussions:

It is well documented that the use of carbon tetrachloride is successfully induce hepatotoxicity in experimental animals due to oxidative damage by free radical generation. Antioxidant property is claimed to be one of the mechanisms of hepatoprotective (Recnagel *et al.*, 1989 and Abd El-Ghany and Nanees, 2010). Silymarin is a powerful antioxidant said to protect liver cells against oxidative damage and other cells in the body from toxins. It also protects liver cells by blocking and removing toxins from the liver. Silymarin aids in regenerating injured liver cells and blocks fibrosis (Pradhan and Girish 2006). It promotes liver cell protein synthesis and decreases the oxidation of glutathione. Silymarin also exerts membrane stabilizing activity and attributes important for liver secretion and uptake of plasma lipoproteins and so alter in lipid metabolism (Vladimir and Daniela 2005).

The improvement of nutritional results of doum groups may be attributed to the energy available from consumption of the edible portions of the nut which is approximately 1300 Kcal/100 g. The kernels were also found to contain crude protein and lipids (Bonde *et al.*, 1990 and Lokuruka 1990). Several fatty acids were identified and isolated from the seeds of doum as caprylic, capric, lauric, myristic, palmitic, stearic, oleic and linoleic while oleic was found to constitute the major fatty contents in the edible part of doum (Hsu *et al.*, 2006). Also, the aqueous extract of doum fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents (Cook *et al.*, 1998).

It is known that Liver enzymes (ALT, AST, γ GT and ALP) activities were used as important biomarkers for detection of hepatotoxicity. The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage or necrosis of hepatocytes. The membrane bound enzymes like ALP and γ GT are released unequally into bloodstream depending on the pathological phenomenon. The co-administration of hepatoprotective agents may induce the hepatocytes to resist the toxic effects of carbon tetrachloride due to its antioxidant property exerted by flavonoids in this plant (Hasan 2011).

Explanation of the possible mechanism underlying the hepatoprotective properties of the doum is having five flavone glycosides were isolated and identified from doum fruits (Cook *et al.*, 1998).

Methionine helps the body process and eliminates fat. It contains sulfur and natural antioxidant, glutathione. The body also needs plenty of methionine to produce two other sulfur-containing amino acids, cysteine and taurine, which help the body eliminate toxins and build strong healthy tissues. One of the important functions of methionine is its ability to be a supplier of sulfur and other compounds required by the body for normal metabolism and growth. Sulfur is a key element and vital to life. Without an adequate intake of sulfur, the body will not be able to make and utilize a number of antioxidant nutrients. Methionine is also a methyl donor for a wide variety of chemical and metabolic reactions inside our body (Sánchez-Góngora *et al.*, 1997 and Parlesak *et al.*, 1998). Methionine is a lipotropic, or a chemical substance that helps the liver process fats (lipids). Other lipotropics include choline, inositol, and betaine (trimethylglycine), all of which help prevent the accumulation of fat in the liver and thus ensure normal liver function, which is essential for the elimination of toxins from the body. Methionine also supports liver function by regulating glutathione supplies which is needed to help neutralize toxins in the liver Methionine is a potent antioxidant and reduces the process of lipid peroxidation (a decreased in the concentration of MDA). The best antioxidative properties have been demonstrated by methionine in rat liver (Bianchi *et al.*, 2000 and Iwona *et al.*, 2009).

Conclusion: It could be concluded that doum powder and extract improve nutritional results and liver functions especially when consumed with methionine which has a best significant treatment effect against hepatotoxicity induced by CCl4 in rats because of its free radical scavenging effect and its ability to increase antioxidant activity.

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