Egypt. J. of Nutrition and Health Vol. 16 No. 2 July (2021)

Effect of Mixture of Chia, Flax and Milk thistle Seeds on Carbon Tetrachloride (CCl_4) Induced Hepatotoxicity in Female Albino Rats.

Asmaa H. Ahmed

Dept. of Home Economics, Faculty of Specific Education
Menoufia University, Egypt.

Abstract

Seeds are known to be a rich house of nutrients despite being small in size. They also contain healthy monounsaturated fats, polyunsaturated fats and many important vitamins, minerals and antioxidants.When consumed as part of a healthy diet, can help reduce blood sugar, liver health,cholesterol and blood pressure. This investigation aimed to study the effect of mixture of chia, flax and milk thistle seeds on carbon tetrachloride (CCl_4) induced liver toxicity in female rats. Twenty female rats weighting (160±5g) were divided into 4 equal groups (n= 5 rats), one group was kept on basal diet as control negative while the other 3 groups were injected by CCl_4 in paraffin oil 50 % v/v (2ml/kg.b.w.t.) twice a week for two weeks to induce liver intoxication; one of them was kept as basal diet and left as control positive (+ve),while the other two groups have the basal diet supplemented with 10 &15 % mixture of chia, flax and milk thistle seeds respectively, Liver function and histology were assessed after 4 weeks. At the end of the experimental period (30 days), blood samples were collected for serum separation to determine serum liver enzymes; Glutamate Oxaloacetate Transaminase (AST), Alanine Amino transferase (ALT) and Alkaline Phosphatase (ALP),total protein, albumin, total cholesterol, triglycerides,lipids profile fractions, antioxidant activities and oestradiol hormone.The obtained results concluded that the mixture of the three seeds at two concentrations 10 and 15% caused a significant improvement in liver enzymes, lipid profile, antioxidant activities and oestradiol hormone ($P \leq 0.05$) in the intoxicated female rats by CCl_4. In conclusion, feeding rats basal diet supplemented with mixture of chia, flax and milk thistle seeds at10,15% have improved in the levels of liver enzymes,lipid profile, antioxidant and oestradiol hormone in female rats with intoxified liver by CCl_4.

Key words:chia seeds - flax seeds - milk thistle seeds - CCl_4- oestradiol hormone.

Introduction

Liver is considered as vital organ of the body, which critically regulates several important body functions including biotransformation and detoxification of the endogenous and exogenous substances ***(Al-Seeniet al.,2016)***. The liver is the storage location of fat-soluble vitamins and handles cholesterol homestasis ***(Latief and Ahmad,2017).*** It stores iron and copper. It plays a role in haematology with clotting factor and protein synthesis, The liver plays a role in heme breakdown into unconjugated bilirubin and conjugates, sex hormone metabolism and produces carrier proteins that are important in reproduction and development. kuffer cells and pit cells play an important role in the body's immunologic system.It may not be surprising to that over 10% of the world population suffers from liver diseases. Most common of these conditions are the hepatitis, hepatic steatosis (fatty liver), fibrosis, cirrhosis, alcoholic and drug induced diseases ***Mustafa et al., (2015).***Currently, many researchers are exploring the plant kingdom in search of novel medicinal agents to alleviate and prevent liver diseases ***(Lopez et al.,2017).*** In addition, amelioration of the chemical's toxicity due to their antioxidant and anti-inflammatory roles as chia, flax and milk thistle seeds ***(Habashyet al.,2021).***

Parkeret al., (2018) reported that Chia (*Salvia hispanica*) is an oilseed plant, which contains natural polyphenols, omega-3 fatty acids mainly α-linolenic acid, high-quality proteins,dietary fibres, vitamins, and minerals. Because of its rich nutritional composition and health benefits, Chia is currently consumed not only as seeds, but also as oil, which brings about similar effects. It has grabbed attention of many food industries and educators and has been used in the production of various food products. Chia can be considered a functional food because of its health beneficial effects to prevent many diseases such as epidemic cardiovascular disease, diabetes, obesity, and related risk factors (***Dikshet al., 2021).***It has been scientifically established that chia is an immune improver and has therapeutic effects on control of anti-inflammatory, diabetes, hypertension, dyslipidaemia,also is important for vision and antioxidant, anti-blood clotting, laxative, antidepressant, antianxiety and analgesic ***(Mburu,2021).***

Shayan et al., (2020) reported that flaxseed (*Linum usitatissimum*) is known as a remarkable functional food with different health benefits.It is a rich source of the omega-3 fatty acid and contains high amounts of alpha-linolenic acid, linoleic acid, lignans, fibre, these compounds provide bioactivity of value to the health of animals and humans through their anti-inflammatory action, anti-oxidative capacity and lipid modulating properties and protects against cardiovascular disease, diabetes, dyslipidaemia, obesity and altogether metabolic syndrome**.**Functional foods are frequently consumed for the prevention and treatment of many diseases. Flaxseed is one of the functional foods with anticancer, antiviral, antibacterial and antifungal properties***(Parikh et al.,2019).***

Millions of people consume milk thistle to support healthy liver function; ***Fanoudi et al.,(2018)*** reviewed that *Silybum marianum* (milk thistle), belongs to the Asteraceae family, possesses different effects such as hepatoprotective, cardioprotective, neuroprotective, anti-inflammatory and anti-carcinogenic activities Several studies have demonstrated that this plant has protective properties against toxic agents and its main component, silymarin, which is the mixture of flavonolignans including silibinin, silydianin and silychristin acts against different biological (mycotoxins, snake venoms, and bacterial toxins) and chemicals (metals, fluoride, pesticides, cardiotoxic, neurotoxic, hepatotoxic, and nephrotoxic agents) poisons.Silymarin is safe in humans at therapeutic doses and is well tolerated even at a high dose of 700 mg three times a day for 24 weeks***(Soleimaniet al.,2019).***

In this study it is aimed to study the effect of mixture of Chia, Flax and Milk thistle Seeds on Carbon Tetrachloride (CCl_4) Induced Hepatotoxicity in Female Albino Rats.

Materials and Methods

Materials:

Seeds and chemicals:

Chia seeds (*Salvia hispanica L.*), Flax seeds (*Linum usitatissimum*) and Milk thistle seeds (*Silybum Marianum)* were obtained from herbalist, Menoufia Governorate,Egypt.Treatments groups were fed on the basal diet containing 10% and 15% of the mixture seeds of chia, flax and milk thistle seeds that were milled to soft powder by using electric grinder and kept in dusky stoppered glass bottles in a cool and dry location till use according to ***Russo and Tyler (2001).***Carbon tetrachloride (CCl_4) and all other chemicals, kits and reagents were obtained from El-Gomhoreya Company, Cairo, Egypt.

Experimental animals:

Mature female albino rats of Sprague-Dawley strain (20 rats) weighting (160±5g) at age of 14 weeks were obtained from the medical insects research institute, Doki, Cairo, Giza, Egypt.

Methods:

Carbon tetrachloride (CCl_4) was dispensed in white plastic bottles each containing one litter as a toxic chemical material.It was mixed with paraffin oil for dilution during the induction.Rats were subcutaneously injected with CCl_4 in paraffin oil (50% v/v) at 2 ml/kg B.Wt. twice in a week for two weeks to induce liver toxicity according to the method described by ***Jayasekharet al., (1997).***

Experimental design:

Twenty female albino rats, Sprague Dawley Strain, 14 weeks age, weighing (160±5g) were used in this experiment. All rats were fed on basal diet according to ***Reeveset al., (1993)*** for 7 days for adaptation. Then rats were divided into four groups (n=5) and fed on the basal diet.First group; negative control (c-ve)group and the other three groups were injected by carbon tetrachloride (CCl_4) twice a week for two weeks to induce hepatotoxicity. The second was positive control (c+ve) group, the third and fourth groups were fed on the basal diet containing 10% and15% of the mixture seeds.

Blood sampling:

After12 hours fasting,at the end of experiment. the rats were scarified under ether anaesthesia. blood samples were collected from hepatic portal vein, blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath (37°C) for 28 minutes, then centrifuged for 10 minutes at 4000 rpm to separate serum, which were carefully aspirated and transferred into clean eppendorf tube and stored frozen at -20°C till analysis as the method prescribed ***Schermer, (1967).***

Biochemical analysis:

Liver enzymes were determined, Glutamate Oxaloacetate Transaminase (AST), Alanine Amino transferase (ALT) and Alkaline Phosphatase (ALP) assayed by the methods of ***Chawla, (2003), Srivastava et al., (2002)and Haussament, (1977)*** respectively. Serum total protein (TP) was carried out according to the colorimetric method of ***Henry, (1974)*** and serum albumin was assessed by ***Doumas et al., (1971*)**. Serum levels of total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL.c) were determined by using the methods of ***Thomas, (1992)*** and ***Fossati and Principe, (1982)*** and ***Grodon and Amer, (1977)*** respectively. The calculation of low-density

lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were carried out according to the methods of ***Lee and Nieman, (1996)*** as follows:
VLDL-c (mg/dl) = Triglycerides / 5 LDL-c (mg/dl) = Total cholesterol – HDL-c – VLDL-c.of antioxidant status in liver,superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPX) were assayed according to the method of ***Sun et al., (1988), Diego, (2011)and Zhao et al., (2001)*** respectively.
Finally, the level of the hormone estradiol in serum was measured according to ***Hinkle and Brunner,(2014).***

Histopathological analysis.

Tissue samples were taken from liver of different groups and used for histopathological examination using H& E stain according to ***Bancroft et al., (1996).***

Statistical Analysis:

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigma Stat, statistical soft-ware, Effects of different treatments were analyzed by one-way ANOVA (Analysis of variance) test using Duncan's multiple range test and p≤ 0.05 was used to indicate significant differences between groups ***(Snedecor and Cochran, 1967).***

Result and Discussion

Data illustrated in table (1) show the effect of mixture of seeds at 10 &15% additives to basal diet on serum liver enzymes including AST,ALT and ALP enzymes of CCl_4intoxicated female rats.It is clear from the table that intoxicated female rats with carbon tetrachloride without treatment (+ve) , the mean ±SD of serum levels of AST, ALT and ALP enzymes were 38.33±5.86, 56.00±6.24 and 174.33±12.9 u/l, respectively, (P≤ 0.05) while in control negative group were 22.66±1.53, 18.66±3.51 and 129.66±4.72, respectively. These data demonstrate significant increases of AST, ALT and ALP enzymes in the serum of female rats control positive (c+ve) compared with normal female rats. Both two groups injected by carbon tetrachloride were fed mixture of Chia, flax and milk thistle seeds 10, 15% showed significant decrease in serum levels of all previous mentioned parameters when compared to control (+ve).
Female rats treated with the mixture of seeds and fed on 15% showed the highest significant decrease in AST, ALT and ALP compared with the other treated group.

Table (1):
Effect of mixture of seeds 10% and 15% fed to intoxicated rats on serum levels of (AST), (ALT) and (ALP) enzymes.

Group	AST u/l	ALT u/l	ALP u/l
G1: Control (-ve)	22.66±1.53^{a}	18.66±3.51^{d}	129.66±4.72^{b}
G2: Control (+ve)	38.33±5.86^{d}	56.00±6.24^{a}	174.33±12.9^{a}
G3: Mixture (10%)	26.66±3.40^{b}	23.33±6.50^{b}	82.00±6.55^{c}
G4: Mixture (15%)	24.66±3.05^{c}	20.00±4.35^{c}	72.66±12.22^{d}

-Values denote arithmetic means ± standard error of the mean.
- Different letters (a, b,c,d, etc.,) in the same column differ significantly at p ≤ 0.05 using one-way ANOVA test, while those with similar letters are not.

Data presented in table(2) show the effect of seeds mixture fed at10% and15% to rats on serum levels of total protein(T.P) and albumin (Alb) denote that the mean value of T.P of (+ve)control group was higher than the (-ve) control group. The treated group showed improved serum T.P when fed on basal diet containing mixture of seeds at 10, 15% when compared to the control (+ve) group.The same table shows that the mean value of Alb of the control (+ve) group was higher than control (-ve) group. Intoxicated female rats fed on basal diet containing 15% mixture of seeds Alb leve improved compared to the (+ve)control group**.**

Table (2):
Effect of mixture of seeds fed at10 and 15% on serum levels (T.P) and (Alb.) in CCl4intoxicated rats.

Group	TP(g/dl)	Alb(g/dl)
G1: Control (-ve)	6.97±0.87 ^{c}	3.93±0.05 ^{b}
G2: Control (+ve)	9.26±2.94 ^{a}	4.80±0.80 ^{a}
G3: Mixture (10%)	7.10±0.87 ^{b}	3.76±0.05 ^{b}
G4: Mixture (15%)	7.73±0.41 ^{b}	3.53±0.63 ^{c}

-Values denote arithmetic means ± standard error of the mean.

- Different letters (a, b,c,d, etc.,) in the same column differ significantly at p ≤ 0.05 using one-way ANOVA test, while those with similar letters are not.

Data presented in table (3) illustrated the effect of seeds on total serum cholesterol (TC) and triglycerides (TG) of intoxicated female rats with CCl_4, it could be noticed that mean value of (TC) and TG of control (+ve) group were higher than control (-ve) group. The best serum (TC) and (TG) levels were shown in group 3 fed on basal diet containing 10% mixture of seeds when compared to control (+ve) group. The same data in table indicated that the mean value of HDLc of the control (+ve) group was lower than the (-ve)control group. The best serum HDLc for group 4 (rats fed on basal diet + 15% mixture of seeds) when compared to the control (+ve) group.The data indicated that mean value of LDLc and VLDLc of control (+ve) group were higher than control (-ve) group.The best serum LDLc and VLDLc were group 3 with treated group 10% mixture of Chia, Flax, Milk thistle seeds when compared to (+ve)control group.

Table (3):
Effect of seeds mixture fed at10 and 15% on serum levels of (TC),(TG) and levels of lipoprotein fractions (HDLc, LDLc and VLDLc)in CCl_4 intoxicatedrats.

Group	Cholesterol Mg/dl	T.G Mg/dl	HDL Mg/dl	LDL Mg/dl	VLDL Mg/dl
G1: Control (-ve)	94.66±5.03^{b}	145.33±3.51^{d}	59.33±5.85 ^{b}	67.33±5.85 ^{c}	27.73±0.70 ^{d}
G2: Control (+ve)	191.33±12.01^{a}	194.66±7.37^{a}	53.00±2.64 ^{d}	71.33±8.62 ^{a}	38.93±1.47 ^{a}
G3: Mixture (10%)	88.00±2.00^{d}	144.3±11.67^{c}	61.66±1.52 ^{a}	68.00±3.60 ^{b}	28.86±2.33 ^{c}
G4: Mixture (15%)	92.66±4.04^{c}	149.00±9.84 ^{b}	54.00±5.00 ^{c}	68.66±2.51 ^{b}	29.8±1.96 ^{b}

-Values denote arithmetic means ± standard error of the mean.

- Different letters (a, b,c,d, etc.,) in the same column differ significantly at p ≤ 0.05 using one-way ANOVA test, while those with similar letters are not.

Table (4) revealed that intoxicated liver caused a significant decrease in CAT, SOD and GPX levels due to injection with CCl_4 when compared to the (-ve) control group.Feeding the seeds mixture 10, 15% as additives to basal diet revealed a significant increase in CAT, SOD and GPX activities compared to the(+ve)control group. The best result was recorded for group 3 rats fed on diet containing 10% of the seeds mixture.

Table (4):
Effect of seeds mixture fed at10 and 15% on antioxidant enzymesCatalyze(CAT), super oxide dismutase (SOD) and glutathione peroxidase (GPX) in CCl_4 intoxicated rats.

Group	CAT (mmo /l)	SOD(u/l)	GPX(Mu/ol)
G1: Control (-ve)	15.80±3.93 [a]	118.85±1.26 [a]	5.42±0.58 [a]
G2: Control (+ve)	5.80±0.95 [d]	92.60±2.39 [c]	4.72±0.43 [c]
G3: Mixture (10%)	10.24±1.18 [c]	117.28±3.03 [a]	5.25±0.55 [b]
G4: Mixture (15%)	11.96±2.69 [b]	115.25±4.05 [b]	5.46±0.49 [a]

-Values denote arithmetic means ± standard error of the mean.
- Different letters (a, b,c,d, etc.,) in the same column differ significantly at $p \leq 0.05$ using one-way ANOVA test, while those with similar letters are not.

Data of table (5) illustrated the effect of seeds mixture on oestradiol hormone in carbon tetrachloride intoxicated female rats. It is clear from the table that there was a significant decreased at control (+ve) group being 174.33±8.55 pg/mlwhile recorded height in normal female rats as recorded92.00±13.45 pg/ml.The best treatment was observed for group 4 where basal diet contained 15% mixture of seed compared with the control (+ve) group.

Table (5):
Effect of seeds mixture fed at 10 and 15% onestradiol hormone in rats infected with CCl_4.

Group	Estradiol
G1: Control (-ve)	174.33±8.55 [a]
G2: Control (+ve)	92.00±13.45 [d]
G3: Mixture (10%)	131.00±16.09 [b]
G4: Mixture (15%)	122.33±4.16 [c]

-Values denote arithmetic means ± standard error of the mean.
- Different letters (a, b,c,d, etc.,) in the same column differ significantly at $p \leq 0.05$ using one-way ANOVA test, while those with similar letters are not.

Results of the present work indicated that the mixture of chia, flax and milk thistle seeds at 10% and 15% additives to basal diet improved all parameters affected by hepatoxicity due to carbon tetra chloride. the current results are in line with previous studies in concern to improvements in liver enzymes, lipid profile, antioxidant activities and oestradiol hormone as well as toxicity of liver.

Data of this study agreed with the finding of ***Fernández et al., (2019)*** who evaluated the effect of chia intake on acute tyloxapol (TI) induced dyslipidaemia, on acute carbon tetrachloride induced steatohepatitis and on mixed damage in non-obese rats.Using chia seeds (*Salvia hispanica L.*) groups were fed a diet with 15% added chia it partially or totally prevented steatohepatitis, and reduced lipids in the dyslipidemic groups. The hypolipidemic and hepatoprotective effects of chia may be correlated to its high content of α-linolenic acid (omega-3) and phenolics. ***Mahfouz, (2020)*** reported that chia seeds contain high amounts of protein, dietary fibre and unsaturated fatty acids. It is also rich in phenolic compounds which are considered as antioxidants. All treated rat groups showed significant decrease in serum (AST, ALT, ALP, GGT), bilirubin, glucose, T. lipids, TC, TG, LDL-c, urea, creatinine, and tumor necrosis factor (TNF, α).

Katunzi - Kilewelaet al., (2021) reported that chia seeds can improve the nutritional content of various food products when they are blended or mixed. Also highlights functional properties of chia seeds, usage in the food industry and fortification of food with chia seeds and concluded using of chia seeds with blending with other food to produce more nutrient dense food products that can be regarded as a functional food.

Sturgeon *et al.,* (2008) indicated that Flaxseed is a rich source of dietary lignans and suggested that dietary flaxseed may modestly lower serum levels of sex steroid hormones, especially in overweight/obese women. Furthermore, ***Andrejčákováet al., (2021)*** reported that feeding flaxseed improved animal behaviour, leakage of enzymes and prevented selected tissue toxic damage induced by xylene by protecting cell membrane integrity and fluidity and by suppressing apoptosis. Furthermore, ***Prasad et al., (2020)*** described that flaxseed which contains high concentrations of α-linolenic acid, has significant lipid lowering effects in animals. and has potent hypolipidemic effects and raises HDL-C. They concluded that flaxseed have significant lipid-lowering effects in animals and humans.These data agree with ***Azzubaidietal., (2019)*** who found a significant increase in liver enzyme levels, total protein, albumin, and globulin.

Trouillaset al., (2008) mentioned that milk thistle has also shown antioxidant properties on hepatocytes. It can inhibit free radicals derived from the metabolism of toxic substances such as ethanol, acetaminophen, and carbon tetrachloride. It stimulates protein synthesis by protecting cell membranes from free radical-induced damage and directly inhibit radical formation. It can also act as a free radical scavenger and increase the intracellular content of scavengers.***Cacciapuotiet al., (2013)*** studies have shown that silymarin increases the activity of superoxide dismutase and serum levels of glutathione and glutathione peroxidase. These finding supported by a clinical trial of ***Jamalian et al., (2020)*** who studied the impact of silymarin on liver oxidative injury at a dose of 280 mg with orally gavage technique to investigate the leucocytosis, liver enzymes levels ,creatine phosphokinase,prothrombin time, partial thromboplastin, international normalized ratio, and white blood cell were measured before and after the intervention, It revealed that in the silymarin group, liver enzymes were lower than the placebo group on the third day after treatment ($P < 0.05$) and Silymarin decreased liver enzymes (ALT, AST, and ALP) and the level of Gamma-glutamyl transferase(GGT) and they recommended to be used in patients with hypoxic liver injury. The results were consistent with ***El-Hassanen, et al., (2021)*** who demonstrated that feeding with milk thistle herb improved, liver functions, kidney functions and lipid profile.

So, further studies should be done to support the relationship of feeding chia, flax and milk thistle seeds to protect and improve health of liver, furthermore Maintaining estrogen signalling to protect against the development of Non-alcoholic fatty liver disease in animal models.

Histopathological Resuts:

Microscopically, liver of rats from group 1(normal group) revealed the normal histological structure of hepatic lobules (photo:1). On contrary, liver of rats from group 2(+ve) control exhibited vacuolardegenerationof hepatocytes, fibroplasia in the portal triad and deposition of brown haemosiderin pigment (photo:2) as well as formation of newly formed bile ductuoles, portal infiltration with inflammatory cells with oval cells proliferation (Photo. 3). However, liver of rats from group 3 fed on basal diet containing 10% mixture of seeds revealed in the portal triad, deposition of brown haemosiderin pigment, Kupffer cells activation, and oval cells proliferation (photo:4). In addition to examined sections from group 4 fed on basal diet containing 15% mixture of seeds showed improved picture, it revealed slight Kupffer cells activation, fibroplasia in the portal triad and oval cells proliferation. Moreover, slight fibroplasia in the portal triad, few portal inflammatory cells infiltration (photo: 5)

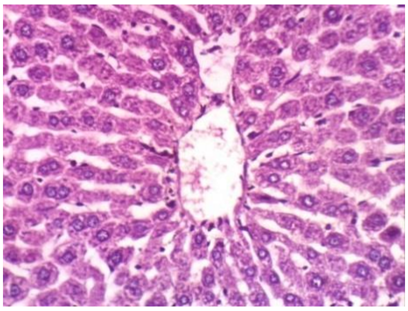

Photo. (1):
Liver of rat from group 1(-ve) female showing the normal histological structure of hepatic lobule (H & E X 400).

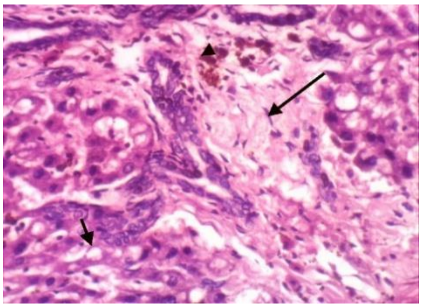

Photo. (2):
Liver of rat from group 2 (+ve) female showing vacuolar degeneration of hepatocytes (small arrow), fibroplasia in the portal triad (large arrow) and deposition of brown haemosiderin pigment (arrow head) (H & E X 400).

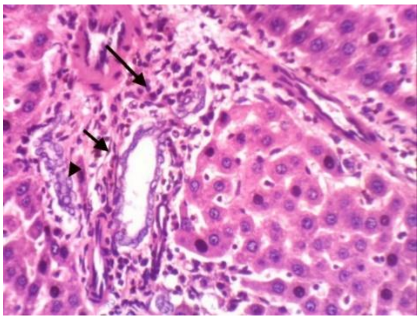

Photo 3:
Liver of rat from group 2 (+ve) female showing fibroplasia in the portal triad and deposition of brown haemosiderin pigment as well as formation of newly formed bile ductuoles, portal infiltration with inflammatory cells with oval cells proliferation (arrow) (H & E X 400**)**.

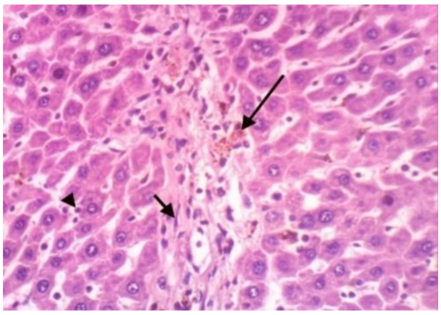

Photo. 4:
Liver of rat from group 3 female showing slight Kupffer cells activation, fibroplasia in the portal triad and oval cells proliferation. Moreover, slight fibroplasia in the portal triad, few portals inflammatory cells infiltration. (arrowhead) (H & E X 400).

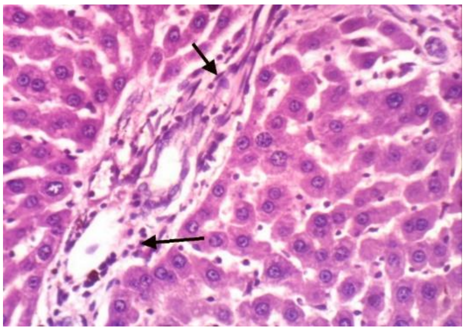

Photo.5:
Liver of rat from group 4 female showing slight fibroplasias in the portal triad (small arrow) and few portals inflammatory cells infiltration (large arrow) (H & E X 400).

Conclusion

Consumption of chia, flax and milk thistle seeds in different doses has valuable effects and protects against hepatoxicity, dyslipidemia, inflammation and some other complications. Mixture of seeds can serve as a promising candidate for the management of metabolic syndrome to control blood lipid levels, liver enzymes and estradiol hormone, Because of their contents of antioxidant activities, flavonolignan, omega-3 fatty acid and contains high amounts of alpha-linolenic acid, linoleic acid, lignans, fibre.

References

Al-Seeni, M.N.; El Rabey, H.A.; Zamzami, M.A. and Alnefayee, A.M. (2016):
The hepatoprotective activity of olive oil and Nigella sativa oil against CCl_4 induced hepatotoxicity in male rats. BMC Complement Altern Med. 4;16(1):438. DOI: 10.1186/s12906-016-1422-4.

Andrejčáková, Z.; Vlčková,R.; Sopková, D. Kozioł, K.; Koziorowski, M.; Fabián, D.; Šefčíková, Z.; Holovská, K.;Almášiová, V. and Sirotkin, A.V. (2021):
Dietary flaxseed's protective effects on body tissues of mice after oral exposure to xylene, Saudi Journal of Biological Sciences. 28(7), 3789-3798. DOI:10.1016/j.sjbs.2021.03.055.

Azzubaidi, M.S.A.; Al-Ahmad, B.E.M. and Dally, I.M. (2019):
The effect of flax seed extract on the blood glucose level and liver bio markers in diabetic rats. World Journal of Pharmacy and Pharmaceutical Sciences. 8 (8), 780-798.

Bancroft, J.D.; Stevens, A. and Turner, D.R. (1996).
Theory and Practiceof Histological techniques. Fourth edition. Churchill Livingstone, New York.

Cacciapuoti, F.; Scognamiglio, A.; Palumbo, R.; Forte, R. and Cacciapuoti, F. (2013):
Silymarin in non-alcoholicfatty liver disease. World J Hepatol. 27;5(3):109-13. DOI: 10.4254/wjh.v5.i3.109.

Chawla, R. (2003).
Practical Clinical Biochemistry. Third Edition. Jaypee Brothers Medical Publichers (p) LTD, New Delhi.

Diego, S. (2011):
OxiselectTM Catalase Activity Assay kit, Colorimetric. Cell Biolabs, Inc., 225(1): 9-22.

Diksh, A. B.; Kamalji, T. K.; Tarvinder, P. S. and Jaspree, T. K. (2021):
Biofortification Strategies For Wheat And Wheat-Based Products. Cereals and Cereal-Based Foods. 1st Edition, Apple Academic Press, 18.

Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1971):

Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 31(1):87-96. DOI: 10.1016/0009-8981(71)90365-2.

El-Hassanen, Y.A.; Badran, H.; Abd EL-Rahman, A.N.A. and Badawy, N.M. (2021):
Potential Effect of Milk Thistle (Silybum Marianum) On Liver Disorders Induced by Carbon Tetrachloride. Journal of Home Economics. 31(1), 83-93.

Fanoudi, S.; Alavi, M.S.; Karimi, G. and Hosseinzadeh, H. (2018):
Milk thistle (*Silybum Marianum*) as an antidote or a protective agent against natural or chemical toxicities: a review. Drug Chem Toxicol. 43(3):240-254. DOI: 10.1080/01480545.

Fernández-Martínez, E.; Lira-Islas, I.G.; Cariño-Cortés, R.; Soria-Jasso, L.E.; Pérez-Hernández, E. and Pérez-Hernández, N. (2019):
Dietary chia seeds (Salvia hispanica) improve acute dyslipidemia and steatohepatitis in rats. J Food Biochem. 43(9):e12986. DOI: 10.1111/jfbc.12986.

Fossati, P. and Principe, L. (1982).
Triglyceride enzymatic colorimetric method. J. of Clin. Chem., 28(10): 2077-2080.

Grodon, T. and Amer, M. (1977).
Determination of HDL. Clin. Chem., 18: 707. (Chemical Kits).

Habashy, N.H.; Kodous, A.S. and, Abu-Serie, M.M. (2021):
Targeting ROS/NF-κBsigaling pathway by the seedless black Vitis vinifera polyphenols in CCl_4-intoxicated kidney, lung, brain, and spleen in rats. Sci Rep. 16;11(1):16575. DOI: 10.1038/s41598-021-96008-0.

Haussament, T. V. (1977).
Determination of alkaline phosphatase. Clin. Chem. Acta, 35: 271 – 273.

Henry, R. J. (1974):
Clinical Chemistry: Principles and Techniques. 2nd Ed, Harper and Publishers, New York Philadelphia.

Hinkle, J. and Brunner, C.K. (2014):
Suddarth's Handbook of Laboratory and Diagnostic Tests. Kindle. 2nd Ed, Kindle. Philadelphia: Wolters Kluwer Health, Lippincott Williams & Wilkins; c2014. Estrogens (Serum) (E1); p. 275-76.

Jamalian, M.; Mahmodiyeh, B.; Saveiee, S. and Solhi, H. (2020):
Investigating the impact of silymarin on liver oxidative injury. J Family Med Prim Care. 2020 Mar 26;9(3):1707-1711. DOI: 10.4103/jfmpc.jfmpc_929_19.

Jayasekhar, P.; Mohanan, P.V. and Rathinam, K. (1997):
Hepatoprotective activity of ethyl acetate extract of Acacia catechu. Indian J Pharmacol. 29:426–8.

Katunzi - Kilewela, A.; Kaale, L.; Kibazohi, O. and Rweyemamu, L. (2021):

Nutritional, health benefits and usage of chia seeds (Salvia hispanica): A review. African Journal of Food Science. 15. 48-59. DOI:10.5897/AJFS2020.2015.

Latief, U and Ahmad, R. (2017):
Herbal remedies for liver fibrosis: A review on the mode of action of fifty herbs. J Tradit Complement Med. 8(3):352-360. DOI: 10.1016/j.jtcme.07.002.

Lee, R. and Nieman, D. (1996):
Nutrition Assessment. 2nd Ed., Mosby, Missouri, USA.

Lopez, C.P.; Sumalapao, D.E.P. and Villarante, N.R. (2017):
Hepatoprotective activity of aqueous and ethanolic Bixa orellana L. leaf extracts against carbon tetrachloride-induced hepatotoxicity. National Journal of Physiology, Pharmacy and Pharmacology, 7: 972-976. DOI: 10.5455/njppp.7.041201105.

Mahfouz, M.Z (2020):
Using Pan Bread Enriched with Chia Seeds to Reduce some Side Effects of Fatty Liver Induced with Fructose in Male Rats. Home economic J. 20, 36(1); 167-204. DOI: 10.21608/jhe.2020.141765.

Mburu, M. (2021):
The Role of Chia Seeds Oil in Human Health. DOI: 10.24018/ejfood.2021.3.4.270

Mustafa, M.E.; Mansoor, M.M.; And Babker, A.A.A. (2015):
Evalution of platelets count and coagulation parameters among patients with liver disease. World J. Pharm. Res., 4(10): 360 -368.

Parikh, M.; Maddaford, T.G.; Austria, J.A.; Aliani, M.; Netticadan, T. and Pierce, G.N. (2019):
Dietary Flaxseed as a Strategy for Improving Human Health. Nutrients. 25;11(5):1171. DOI: 10.3390/nu11051171. PMID: 31130604; PMCID: PMC6567199.

Parker, J.; Schellenberger, A.N.; Roe, A.L.; Oketch-Rabah, H. and Calderón, A.I.(2018):
Therapeutic Perspectives on Chia Seed and Its Oil: A Review. Planta Med. 84(9-10):606-612. DOI: 10.1055/a-0586-4711.

Prasad, K.; Khan, A.S. and Shoker, M. (2010):
Flaxseed and Its Components in Treatment of Hyperlipidemia and Cardiovascular Disease. Int J Angiol. 29(4):216-222. DOI: 10.1055/s-0040-1709129.

Reeves, P.G.; Nielsen, F.H. and Fahey, G.C. (1993):
AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 123(11):1939-51. DOI: 10.1093/jn/123.11.1939.

Russo, E. and Tyler, V.M. (2001):
Handbook of Psychotropic Herbs: A Scientific Analysis of Herbal Remedies for Psychiatric Condition. Howrth Herbal Press, USA.

Schermer, R.M. (1967).
Radio protective effects of Mentha piperita, J Med Arom Plant Sci., 23(1A): 91-97.

Shayan, M.;Kamalian, S.;Sahebkar, A. andTayarani-Najaran, Z. (2020):
Flaxseed for Health and Disease: Review of Clinical Trials. Comb Chem High Throughput Screen. 23(8):699-722. DOI: 10.2174/1386207323666200521121708.

Snedecor, G. W. and Cochran, W. G. (1967):
Statistical Methods. 6th Ed. Iowa State University Press. Ames. Lowa. USA.

Soleimani, V.; Delghandi, P.S.; Moallem, S.A. and Karimi, G. (2019)
Safety and toxicity of silymarin, the major constituent of milk thistle extract: An updated review. Phytother Res. 33(6):1627-1638. DOI: 10.1002/ptr.6361.

Srivastava, L. M.; Das, N. and Sinha, S. (2002):
Essentials of Practical Biochemistry. CBC Publishers and Distributors.

Sturgeon, S.R.; Heersink, J.L.; Volpe, S.L.; Bertone-Johnson, E.R.; Puleo, E.; Stanczyk, F.Z.;Sabelawski, S.;Wahala, K.; Kurzer, M.S. and Bigelow, C. (2008):
Effect of dietary flaxseed on serum levels of estrogens and androgens in postmenopausal women. Nutr Cancer. 60(5):612-8. DOI: 10.1080/01635580801971864.

Sun, Y.; Oberley, L.W. and Li, Y. (1988):
A simple method for clinical assay of superoxide dismutase. Clin Chem. 34(3):497-500. DOI: 10.1093/clinchem/34.3.497

Thomas, L. (1992):
Labor and Diagnose, 4th Ed., (Chemical Kits).

Trouillas, P.; Marsal, P.; Svobodová, A.; Vostálová, J.; Gazák, R.; Hrbác, J.; Sedmera, P.; Kren, V.; Lazzaroni, R.; Duroux, J.L. and Walterová, D. (2008):
Mechanism of the antioxidant action of silybin and 2,3-dehydrosilybin flavonolignans: a joint experimental and theoretical study. J Phys Chem A. 7;112(5):1054-63. DOI: 10.1021/jp075814h.

Zhao, Y.; Kiningham, K.K.; Lin, S.M. and St-Clair, D.K. (2001):
Overexpression of MnSOD protects murine fibrosarcoma cells (FSa-II) from apoptosis and promotes a differentiation program upon treatment with 5-azacytidine: involvement of MAPK and NFkappaB pathways. Antioxid Redox Signal. 3(3):375-86. DOI: 10.1089/15230860152409022.

تأثير خليط بذور الشيا والكتان وحليب الشوك على السمية الكبدية التي يسببها رابع كلوريد الكربون في إناث الجرذان البيضاء

د. أسماء حسن عبد العظيم أحمد

مدرس التغذية وعلوم الأطعمة - قسم الاقتصاد المنزلي - كلية التربية النوعية - جامعة المنوفية – مصر

الملخص العربى

من المعروف أن البذور مصدر غني بالمواد الغذائية على الرغم من صغر حجمها. كما أنها تحتوي على الدهون الصحية الأحادية غير المشبعة والدهون المتعددة غير المشبعة والعديد من الفيتامينات والمعادن ومضادات الأكسدة الهامة، وعند تناولها كجزء من نظام غذائي صحي، يمكن أن تساعد في تقليل نسبة السكر في الدم وصحة الكبد والكوليسترول وضغط الدم ، هدفت هذه الدراسة إلى دراسة تأثير خليط بذور الشيا والكتان وحليب الشوك على رابع كلوريد الكربون ($CCl4$) المسبب لتسمم الكبد في إناث الجرذان. تم تقسيم عشرين من إناث الجرذان أوزانهم (160 ± 5 جم) إلى 4 مجموعات متساوية (ن = 5 جرذ)، مجموعة واحدة بقيت سالبة بينما تم حقن الثلاث مجموعات الأخرى بواسطة(CCl_4) في زيت البرافين بنسبة 50٪ حجم /2 مل/ كجم مرتين في الأسبوع لمدة أسبوعين لحدوث تسمم الكبد. تركت إحداهما كمجموعة ضابطة موجبة بينما اتبعت المجموعتان الأخريان نظامًا غذائيًا أساسيًا مكملًا بمزيج 10 و15٪ من بذور الشيا والكتان وحليب الشوك، وتم تقييم وظائف الكبد والأنسجة بعد 4 أسابيع في نهاية الفترة التجريبية (30 يومًا)، تم جمع عينات الدم لفصل المصل لتحديد إنزيمات الكبد في الدم، البروتين الكلي،الألبومين، الكوليسترول الكلي، الدهون الثلاثية،ونشاط مضادات الأكسدة وهرمون الاستراديول: خلصت النتائج المتحصل عليها: إلى أن خليط البذور الثلاثة السابقة بتركيزي 10،15٪ أدى إلى تحسن معنوي في إنزيمات الكبد، ونسبة الدهون، وأنشطة مضادات الأكسدة وهرمون الاستراديول ($P \le 0.05$) في إناث الجرذان المصابة برابع كلوريد الكربون ، في الختام ، فإن تغذية خليط بذور الشيا والكتان وحليب الشوك المكملة للنظام الغذائي الأساسي بنسبة 10،15 ٪ لها دور إيجابي في تعزيز مستويات إنزيمات الكبد والدهون ومضادات الأكسدة و هرمون الاستراديوللدى إناث الجرذان المصابة بالتسمم الكبدي برابع كلوريد الكربون .

الكلمات المفتاحية: بذور الشيا - بذور الكتان - بذور حليب الشوك - رابع كلوريد الكربون - هرمونالاستراديول.