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Functional foods extracts app lied in breads ameliorates liver disorders induced by CCl_4 in rats

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Abstract: Liver cancer is one of the most common cancers representing a leading cause of cancer related mortality, with an increasing incidence worldwide. Several strategies to fight cancer and its complications have been proposed, because early discovering, prevention and treatment play a pivotal role in reducing the population burden of cancer. Therefore, the present study was designed to determine the effects of five functional foods of plant source extracts (black caraway seeds, barley wheat, oat wheat, turmeric rhizome and gum Arabic and their mixture bended in bread on liver disorders-induced by CCl_4 in rats. Treatment of animals with CCl_4 caused a significant increased ($p\leq0.05$) in serum ALT, AST, ALP and total bilirubin by the ratio of 142.72, 238.21, 37.02 and 568.18% compared to normal controls. Supplementation of the rat diets with pan bread blending with 0.1% w/w of black-caraway seeds methanol extract (BCSME), barely flour methanol extract (BFME), oat flour methanol extract (EPME), turmeric rhizome methanol extract (TRME), gum arabic water extract (GAWE) and their mixture significantly ($p\leq0.05$) decreased these parameters by different ratios. The same behavior was recorded for MDA level in red blood cells, the biomarkers of oxidative stress in tissues. These results supported our hypothesis that such functional foods extract contains several classes of phytochemicals with other compounds that are able to prevent or inhibit CCl_4 hepatotoxicity through liver serum enzymes-lowering activity, decreasing rate on the formation of MDA erythrocytes. Therefore, we recommended like of that functional foods extract by a concentrations ranged 0.1% amount to be included in our daily diets, drinks, food supplements/ additives etc.

Keywords: black-caraway seeds, barley flour, oat flour, turmeric, gum Arabic, liver functions, bilirubin, MDA.

Introduction

Liver is a vital organ present in all vertebrates. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemical necessary for digestion (Voet and Voet, 1990 and Elhassaneen, 1996). Liver cancer is one of the most common cancers representing a leading cause of cancer related mortality, with an increasing incidence worldwide, being responsible for more than one million deaths annually (Lee *et al.,* 2005).

Several strategies to fight cancer and its complications have been proposed, because early discovering, prevention and treatment play a pivotal role in reducing the population burden of cancer. Benefits of pharmaceutical factors to treat the disease aggressively early have been recommended, but medications may have unwanted side effects. Also, the cost of administrating modern anticacinogenic drugs is beyond the reach of most people in the low income group and those living in the rural areas. Thus, the therapeutic approach of several traditional medicinal systems is more holistic. Historically, most drugs have been derived from natural products, but there has been a shift away from their use with the increasing predominance of molecular approaches to drug discovery (Harvey, 1999). Among chemotherapeutic or chemo-preventive natural sources, functional foods come as a good protector being a natural product. They are similar in appearance to conventional foods; the former being consumed as part of the normal diet. In contrast to conventional foods, functional foods, however, have demonstrated physiological benefits and can reduce the risk of chronic disease beyond basic nutritional functions, including maintenance of gut health. When food is being cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called "functional food". Thus, functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, etc., needed for its healthy survival (FAO, 2010). According to the definition, functional food is a part of an everyday diet and is demonstrated to offer health benefits and to reduce the risk of chronic disease beyond the widely accepted nutritional effects. The functional foods comprise: (i) conventional foods containing naturally occurring bioactive substances (e.g., dietary fiber), (ii) foods enriched with bioactive substances (e.g., probiotics, antioxidants), and (iii) synthesized food ingredients introduced to traditional foods (e.g., prebiotics) (Grajek *et al.*, 2005).

Many of authorities and academic centers of research pay more attention towards the area of functional foods in relation to cancer. Scientists have identified thousands of bioactive compounds including flavonoids, glucosinolates (isothiocyanates and indoles), phenolic acids, phytates, and phytoestrogens (isoflavones and lignans), in functional foodsof plant sources. A vast variety of these compounds that are present in the daily human diet have been found to possess substantial anticarcinogenic properties (Surh, 2002). The preventive effects of the majority of edible bioactive compounds are often attributed to their antioxidative or anti-inflammatory activities (Surh *et al.*, 2001). We will restrict our research here on five functional foods of plant source including, black caraway seeds, barley wheat, oat wheat, turmeric rhizome and gum Arabic. Such foods are commonly distributed in the Arab countries including Egypt.

Black caraway *(Nigella sativa* L., Family *Ranunculaceae)* is a herbal plant which is popularly called the seed of blessing (Habatul-barakah) in Arabic countries. Seeds of black caraway contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%). Furthermore, traces of alkaloids are found which belong to two different types: isochinoline alkaloids and pyrazol alkaloids (Tembhurne et al., 2014). The seeds have traditionally been used for thousands of years in the Middle East, Far East and Asia as a food additive and as a herbal health aid (Halawani, 2009). It is suggested that black seed, its oil and extracts act as antimicrobial, immune stimulant, hypotensive, anti-inflammatory, anticancer, antioxidant, hypoglycemic, spasmolytic and bronchodilator (Boskabady *et al.,* 2008; Abel-Salam, 2012 and Lei *et al.,* 2012).

Oat (*Avena sativa*) and barley (*Hordeum vulgare*), although consumed in considerably lower quantities than rice and wheat, have the advantage that they are normally consumed as whole grain cereals and considered as a ‘health food’ for humans. Their whole grains are also good potential sources of fiber, vitamins, minerals and bioactive compounds such as phenolics, carotenoids, vitamin E, phytic acid, β-glucan and sterols. Such bioactive compounds may provide desirable health benefits beyond basic nutrition, such as a reduced risk of chronic diseases such as coronary heart diseases (Adil, 2012).

Turmeric (*Curcuma longa L.*) belongs to the *Zingiberaceae* family along with the other noteworthy members like ginger, cardamom and galangal. Turmeric contains a wide variety of phytochemicals, including but not limited to curcumin, zingiberene, curcumenol, curcumol, eugenol, turmerin, turmerones, and turmeronols (Chattopadhyay *et al.,* 2004). Traditionally many medicinal properties are attributed to this spice. Since the time of 1900 BC numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders (Aggarwal *et al.,* 2007).

Gum Arabic (GA) or Acacia gum is an edible biopolymer obtained as exudates of mature trees of *Acacia senegal* and *Acacia seyal* which grow principally in the African region of Sahe in Sudan. The effective biological role of GA has confirmed in the last twenty years including reduction in plasma cholesterol level in animals and humans (Tiss *et al.,* 2001), anticarcinogenic effect (Nasir *et al.,* 2010) and antioxidant effect (Ali *et al.,* 2003; and Ali and AlMoundhri, 2006) with a protective role against hepatic and cardiac toxicities. In addition to that, it has been claimed that GA alleviates effects of chronic renal failure in humans (Ali *et al.,* 2010). Also, GA can serve to reduce obesity and therefore prevent associated complications in humans including coronary heart disease, stroke and diabetes (Lear *et al.,* 2003 and Hedley *et al.,* 2004).

According to our knowledge, the studies regarding the potential effects of such functional foods on liver disease/cancer are so limited. Therefore, the present study was designed to determine the effects of five functional foods of plant source extracts (black caraway seeds, barley wheat, oat wheat, turmeric rhizome and gum Arabic and their mixture bended in bread on liver disorders-induced by CCl_4 in rats.

Materials and Methods

Materials

Functional foods: Black-caraway (*Nigella sativa*), oat (*Avena sativa L.*), *barley* (*Hordeum vulgare* L.) and *turmeric* (*Curcuma longa*) were obtained with special arrangements from the Shebin El-Kom City, Minoufiya Governorate, Egypt. Gum Arabic (*Acacia senegal* L.) was obtained from the SAVANNA Companies Group (Processing Gums, Juices and Confectionery), Khartoum, Sudan. (Specification: appearance colour off white, appearance form- powder, purity 99.2%).

Chemicals: Carbon tetrachloride (CCl_4) was obtained from El-Gomhoryia Company for Drugs, Chemicals and Medical Instruments Trading, Cairo, Egypt as 10% liquid solution. It was dispensed in white plastic bottles each containing one liter as a toxic chemical material for liver poisoning according to Passmore and Eastwood (1986). In the same time, it was mixed with olive oil which obtained from the pharmacy for dilution during the induction. Casein, as main source of protein from Morgan Company for Chemicals. Cairo, Egypt. Vitamins and salts mixtures components, all organic solvents and other chemicals were of analytical grade were purchased from El-Ghomhorya Company for Drugs, Chemicals and Medical Instruments Trading, Cairo, Egypt.

Pan bread components: Wheat (*Triticum vulgare*) flour, sugar (sucrose) , skim milk powder, baking powder processed by TAG EL MELOUK Co. for food industries, 6^{th} October, Egypt, salt, processed by El-Nasr Salines Co., Alexandria, Egypt. All of these were obtained from Shebin El-Kom market, Menoufiya Governorate, Egypt.

Methods

Preparation of functional foods powder and extracts

Black-caraway, oat, barley and turmeric dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 55 ^{0}C until arriving by the moisture in the final product to about 8%. The dried samples and gum arabic were ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use. Powders of the selected functional foods were used for their different types extracts according to the methods of Amin *et al.,* (2004) and Elmaadawy, (2016).

Processing of pan bread

Pan bread was prepared according to the method described in AACC, (2000). Pan bread making involved mixing 100 g flour, sugar (4% w/w), fat (4% w/w), skim milk powder (2% w/w), salt (1.5% w/w) and water. Bread doughs were prepared by mixing all ingredients in a suitable bowl until they reached maximum development. The resulted dough's were let to rest for 20 min at 28 - 30˚C (first proofing) then the doughs were divided into three 150 g pieces, hand-moulded and put into pans for final proofing at 32 - 35˚C and 80 - 85% relative humidity in fermentation cabinet for 60 min, then baked in electrically heated oven with steam added during baking at 210 - 220˚C for 15 - 20 min. After baking, loaves were separated from the metal pan and allowed to cool at room temperature before sealed in polyethylene bags to prevent moisture loss then stored at room temperature (18±2˚C). Another pan bread making by using functional foods extracts i.e. black-caraway, oat, *barley*, turmeric, gum Arabic and their mixture (by equal parts) by the ratio of 0.1%

Biological experimental

Animals

Mature male albino rats of Sprague - Dawley strain rats weighing (190±20) g were used in this work. Animals were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. The animals were allocated in plastic cages with metallic stainless covers and kept under strict hygienic measures. Rats were fed on basal diet for 7 days before the beginning of the experiment for adaptation. Diets were presented to rats in special non-scattering feeding cups to avoid loss of food and contamination. Water and food was provided *ad libitum* via a narrow mouth bottle with a metallic tube tightly fixed at its mouth by a piece of rubber tube.

Basal Diet

The basal diet was prepared as follow: 20% protein (casein), 10% sucrose, 4.7% corn oil, 0.2% choline chloride, 0.3 % methionin 1% vitamin mixture, 3.5% salt mixture and 5% fiber (cellulose). The remainder was corn starch, 55.3%. Salt mixture and vitamins mixture were prepared according to Hegested *et al.,* (1941) and Campbell, (1963).

Experimental design

All biological experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats (n=54 rats), were housed individually in wire cages in a room maintained at 25 ± 2 ^{0}C and kept under normal healthy conditions. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1, 6 rats) still fed on basal diet and the other main group (48 rats) was injected subcutaneous by carbon tetrachloride to induce liver disorder rats then classified into seven sub groups as follow:

- Group (2): Fed on standard diet only as a positive control (rats with liver disorders).
- Group (3): Fed on standard diet containing 20% pan bread (PB) without functional foods extracts.
- Group (4): Fed on standard diet containing 20% PB with 0.1 % (w/w) Black-caraway seeds methanol extract (BCSME).
- Group (5): Fed on standard diet containing 20% PB with 0.1 % (w/w) oat flour methanol extract (OFME).
- Group (6): Fed on standard diet containing 20% PB with 0.1 % (w/w) barley flour methanol extract (BFME).
- Group (7): Fed on standard diet containing 20% PB with 0.1 % (w/w) turmeric rhizomes methanol extract (TRME).
- Group (8): Fed on standard diet containing 20% PB with 0.1 % (w/w) gum Arabic methanol extract (GAME).
- Group (9): Fed on standard diet containing 20% PB with 0.1 % (w/w) mixture, BCSME+OFME+BFME+TRME+GAME by equal parts.

At the end of experiment period, 28 days, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into glass centrifuge tubes, containing oxalate solution (1.34 %) as anticoagulant. After centrifugation at 3000 rpm for 10 min., plasma was with drown and used for the analysis of blood lipid parameters. The erythrocyte residue was washed with three successive portions of sodium chloride solution (0.9 %) and then haemolysed with deionised water for 30 min. Haemolysate was then centrifuged at 30,000 rpm for 30 min. and the supernatant fractions was transferred to a clean test tube and analyzed of MDA (Stroev and Makarova, 1989).

Biochemical analysis

Liver functions

Alkaline phosphatase (ALP) and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to IFCC, (1983) and Yound (1975), respectively. Serum total bilirubin was determined colorimetrically as described by Doumas *et al.*, (1973).

Oxidative stress (Malonaldialdehyde content, MDA)

Lipid peroxide levels measured as malondialdehyde in liver were determined by as thiobarbituric acid reactive substances (TBARS) as described by Stroev and Makarova, (1989). Half milliliter of homogenate were added to 1.0 ml of thiobarbituric acid reagent, consisting of 15% TCA, 0.375% thiobarbituric acid (TBA) and 0.01% butylated hydroxytoluene in 0.25 N HCl. Twenty-five microliters of 0.1 M $FeSO_4.7H_2O$ was added and the mixture was heated for 20 min in boiling water. The samples were centrifuged at 1000 rpm for 10 min and the absorbance was read at 535 nm using Labo-med. Inc., spectrophotometer against a reagent blank. The absorbance of the samples was compared to a standard curve of known concentrations of malonaldialdehyde.

Statistical analysis

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, statistical soft-ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p \leq 0.05$ was used to indicate significance between different groups **(Snedecor and Cochran, 1967).**

Results and Discussions

Effect of functional foods applied in pan bread on liver functions of rats treated with CCl_4

Data in table (1) represented the effect of feeding functional foods extracts i.e. BCSME,TRME, BFME, OFME, GAWE and their mixture by ratio the ratio of 0.1% (w/w) respectively on serum liver enzymes including AST, ALT and ALP of rats treated with CCl_4. From such data it could be noticed that treatment of rats with CCl_4 caused a significantly ($P \leq 0.05$) increased in AST, ALT and ALP by the percent of change 142.72, 238.21 and 37.02%, respectively compared to normal control. The data showed that there were significantly ($P \leq 0.05$) decreased in AST, ALT and ALP for BCSME,TRME, BFME, OFME, GAWE and their mixture compared to control (-) group by the ratio of 53.54, 80.91, 96.77, 50.28, 56.23 and 45.09%; 39.63, 61.43, 81.17, 37.15, 48.06 and 26.45%; and 9.45, 20.75, 22.12, 11.82, 15.90 and 8.30%, respectively.

Toxic effects of CCl_4 on liver have been extensively studied (Amin and Mahmoud, 2009; Kim *et al.*, 2010). Serum AST and ALT activities are the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar, 2002). During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage (Pari and Murugan, 2004). Serum ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, serum ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (Williamson *et al.*, 1996). In conjunction with the reports of Hegde and Joshi, (2009) and Kim *et al.*, (2010), data from the present study showed that CCl_4 caused hepatic damage with a significant increase in serum levels of AST and ALT. Serum ALP level is also related to the status and function of hepatic cells. CCl_4 administration in the present study also caused significant increase in the serum ALP which may be due to increased synthesis in presence of increasing biliary pressure (Moss and Butterworth, 1974). These results are in accordance with those of Murayama *et al*. (2008) and Abdalla *et al*. (2013). It is clear that the increased enzymatic activities level of ALT was the most affected enzyme meanwhile ALP was the least one. Lin *et al*. (1997) demonstrated that ALT is more sensitive test for hepatocellular damage than AST. CCl_4 impair the integrity, structure and function of the hepatocytes via its ROS, leading to defective secretion of the bile due to the damaged bile ducts consequently elevation of ALP level in the blood (Sreelatha *et al*., 2009).

Our emerged data revealed that functional foods extracts i.e. BCSME,TRME, BFME, OFME, GAWE and their mixture significantly ameliorated the increase of hepatic enzymes AST, ALT and ALP activities levels. The hepatoprotective action of BCSME could be attributed to its bioactive compounds content such as thymoquinone (TQ) (Tembhurne *et al.*, 2014). Its protective action against the hepatotoxin: terbutyl hyderoperoxide has been demonstrated using isolated rat hepatocytes. In that study, the hepatoprotective activity of TQ was compared with that of silybin a known hepatoprotective agent. The mechanism of hepatoprotection of TQ is not certain but may be related to the preservation of intracellular gluathione (GSH), the depletion of which by oxidative stress is known to increase the susceptibility of cells to irreversible injury. It has also been shown that pretreatment of rats with black caraway seeds oil for 4 weeks was effective in protection against CCl_4 and *D*-galactosamine induced hepatic damage. No ill effects on liver function were observed when the oil was green orally at a dose of 100 mg/kg/day for 4 weeks.. Also, it is also found to show protective effects against ischemia reperfusion injury on liver (Fahrettin *et al.,* 2008). The effect of both BFME and OFME, as hepatoprotective agents could be attributed to their presence of the lunasin, bioactive compound, a novel 4.8 kDa cancer preventive seed-peptide, which has been reported to be prevalent in barley and oat (Jeong *et al.,* 2002). Lunasin was also seen to inhibit histone acetylation in mouse fibroblast and human breast cells. In another report, the bioavailability of lunasin *in vivo* has been studied [90]. Oral administration is an important feature of an ideal cancer preventive agent. The liver and kidney of rats fed with barly/oat showed the presence of lunasin in Western Blot (Nirupama *et al.,* 2015). For TRME, Curcumin (the main colored/ active responsible compound) restored the level of biomarker parameters such as AST, ALT and ALP back to normal (Swamy *et al*., 2012). Also, Al-Kenanny *et al*., (2012) studied that received of GA orally to mice at concentration 10g/kg/for eight days, have ability as antioxidant because have a significantly ameliorating hepatotoxicity by increase the level of GSH (biologic antioxidant) and reduction MDA (oxidative stress biomarker) in addition to enzymatic level ALT and AST in serum of mice although it haven't reach to normal value. The rate of suppression was increased with the mixture treatment gave maximum reduction yield of liver functions enzymes activities when compared with the tested function foods separated. It could be mean that a combination of different functional foods may be more efficient for reducing serum level of AST, ALT and ALP, the biomarkers of liver functions stress, because the interactive effects occurred by different categories of bioactive compounds content of functional foods used.

Effect of functional foods applied in pan bread on total bilirubin of rats treated with CCl_4

Data in Table (2) represented the effect of feeding functional foods extracts i.e. BCSME,TRME, BWME, OWME, GAWE and their mixture by ratio the ratio of 0.1% (w/w) respectively on total bilirubin of rats treated with CCl4. From such data it could be noticed that treatment of rats with CCl_4 caused a significantly ($P \leq 0.05$) increased in total bilirubin by the percent of change 568.18%, respectively compared to normal control. The data showed that there were significantly ($P \leq 0.05$) decreased in serum total bilirubin for BCSME,TRME, BWME, OWME, GAWE and their mixture compared to control (-) group by the ratio of 90.91, 181.82, 213.64, 154.55, 104.55 and 63.64%, respectively.

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical (Johnson and Kroening, 1998). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl_4 (Kaplowitz *et al*., 1986). Also, CCl_4 impair the integrity, structure and function of the hepatocytes via its ROS, leading to defective secretion of the bile due to the damaged bile ducts consequently elevation of ALP level in the blood (Sreelatha *et al*., 2009).This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP. These results are in agreement with Ghanem *et al*., (2009) who attributed this reduction in serum total bilirubin to cynarin

Table 2. Effect of functional foods applied in pan bread on total bilirubin (mg/dL) of rats treated with CCl_4

Groups	Mean ±SD	% of change
Control (-)	0.22±0.03 [d]	------
Control (+)	1.47±0.08 [a]	568.18
Pan bread (PB)	1.21±0.10 [a]	450.00
PB+BCSME	0.42±0.20 [bc]	90.91
PB+BFME	0.62±0.11 [b]	181.82
PB+OFME	0.69±0.23 [b]	213.64
PB+TRME	0.56±0.10 [bc]	154.55
PB+GAWE	0.45±0.11 [bc]	104.55
Mixture	0.36±0.17 [bc]	63.64

BCSME, Black caraway seeds methanol extract, BFME, Barley flour methanol extract, OFME, Oat flour methanol extract, TRME, Turmeric rhizome methanol extract, GAWE, Gum Arabic water extract and mixture, mixture of BCME+ BFME+ OFME+ TRME and GAME by equal parts. Each value represents mean ± SD. Means in the same row with different litters are significantly different at $p < 0.05$.

which stimulates the clearance of bile from the liver, preventing congestion in the liver and diminishing the liver damage in protein.

Data of the present study are in agreement with Sylvain *et al.,* (1999) and Obianime and Uche, (2008) who attributed this reduction in serum bilirubin to the bioactive compounds found in the tested functional foods which stimulates the clearance of bile from the liver, preventing congestion in the liver and diminishing the liver damage. The rate of improvement was increased with the mixture treatment gave maximum reduction yield of serum total bilirubin when compared with the tested function foods separated. It could be mean that a combination of different functional foods may be more efficient for reducing serum total bilirubin, the biomarkers of liver functions stress, because the interactive effects occurred by different categories of bioactive compounds content of functional foods used.

Effect of functional foods applied in pan bread on erythrocytes lipid peroxidation (Malondialdehyde, MDA) of rats treated with CCl_4

Data in Table (3) represented the effect of feeding functional foods extracts i.e. BCSME,TRME, BWME, OWME, GAWE and their mixture by ratio the ratio of 0.1% (w/w) respectively on lipid peroxidation (malondialdehyde, MDA) of rats treated with CCl_4. From such data it could be noticed that treatment of rats with CCl_4 caused a significantly ($P \leq 0.05$) increased in MDA, by the percent of change 66.36%, respectively compared to normal control. The data showed that there were significantly ($P \leq 0.05$) decreased in erythrocytes MDA for BCSME,TRME, BWME, OWME, GAWE and their mixture compared to control (-) group by the ratio of 28.08, 32.47, 34.48, 25.46, 22.59 and 18.02%, respectively. The highest suppression was recorded with the mixture treatment. It could be mean that a combination of different plant parts may be more efficient for reducing liver tissue MDA level, the biomarkers of oxidative stress and inflammation in liver, because the interactive effects occurred by different categories of bioactive compounds of functional foods used.

Since the erythrocytes oxidative stress increased in human patients with severe hepatic disease (Solov'eva, 2009). So, the current investigation studied the erythrocytes oxidative stress as a consequence of hepatic injury induced by CCl_4. Oxidative stress in the erythrocytes in the present investigation can be assessed by induction in MDA content. In conjunction with the earlier study of Eritsland (2000), erythrocytes may be prone to oxidative stress because they exposed to high oxygen tension, have polyunsaturated fatty acids in the membrane and hemoglobin bound iron. Antioxidant and anti-inflammatory agents play a critical role against CCl_4 intoxication by scavenging active oxygen and free radicals and neutralizing lipid peroxides (Gutierrez and Navarro, 2010). Malondialdehyde shows both mutagenic and carcinogenic effects by changing membrane properties (Gerbhart, 2002). Lipid peroxidation usually results in decreasing membrane fluidity, cell injury and may cause the formation of atherosclerotic plaques (Kris *et al.*, 2002). Cross linking with the membrane components, MDA causes inactivation of enzymes and receptors in membranes and thus changes membrane properties. Malondialdehyde also causes mutations by reacting with guanine nucleotide in DNA (Cline *et al.*, 2004). Thus, the estimation of free radical activity was done through the determination of malondialdehyde (MDA) which is a by-product of lipid peroxidation.

Table 3. Effect of functional foods applied in pan bread on erythrocytes lipid peroxidation [Malondialdehyde (MDA), nmol/mg Hb] of rats treated with CCl_4

Groups	Mean ±SD	% of change
Control (-)	230.16±4.78 [d]	------
Control (+)	382.9±5.11 [a]	66.36
Pan bread (PB)	359.11±11.56[a]	56.03
PB+BCSME	294.80±10.52 [b]	28.08
PB+BFME	304.89±9.98 [b]	32.47
PB+OFME	309.53±7.13 [b]	34.48
PB+TRME	288.77±5.62 [bc]	25.46
PB+GAME	282.15±4.99 [bc]	22.59
Mixture	271.63±8.03 [bc]	18.02

BCSME, Black caraway seeds methanol extract, BFME, Barley flour methanol extract, OFME, Oat flour methanol extract, TRME, Turmeric rhizome methanol extract, GAWE, Gum Arabic water extract and mixture, mixture of BCME+ BFME+ OFME+ TRME and GAME by equal parts. Each value represents mean ± SD. Means in the same row with different litters are significantly different at $p < 0.05$.

The decreasing in serum MDA levels as the result of feeding functional foods extracts including BCSME, BWME, OWME, TRME and GAME could be attributed to their higher content of phytochemicals.

Several reports have documented the potent antioxidant capacity of curcumin where by mitigation of lipid peroxidation and oxidative stress in several tissues were demonstrated (Nabavi *et al*., 2012). Also, cereal based diets including oat and barley modulate some markers of oxidative stress such MDA and inflammation in lean and obese Zucker rats (Damien *et al.*,2011). For GA, Al-Kenanny *et al*., (2012) studied that received of GA orally to mice at concentration 10gm /kg/for eight days, have ability as antioxidant because have a significantly ameliorating hepatotoxicity by increase the level of GSH and reduction MDA. Also, Badreldin *et al*., (2009) who indicated that gum arabic is a strong antioxidant so it has a protective effect against lipid peroxidation and Said *et al*. (2014) showed that statistical significant decrease of oxidants parameters such as MDA and nitric oxide is as a result of administration of GA with indomethacin because it has free radical scavenging properties.

In conclusion, functional foods extract including BCSME, BWME, OWME, TRME and GAME were effective in protecting against CCl_4-induced liver injuries. These results supported our hypothesis that such functional foods extract contains several classes of phytochemicals with other compounds that are able to prevent or inhibit CCl_4 hepatotoxicity through liver serum enzymes-lowering activity, hypoglycemic effect, decreasing rate on the formation of MDA erythrocytes. Therefore, we recommended like of that functional foods extract by a concentrations ranged 0.1% amount to be included in our daily diets, drinks, food supplements/additives etc.

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إضافة مستخلصات النباتية الى الخبز لمعالجة أضرار الكبد المستحثة برابع كلوريد الكربون فى الفئران

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الملخص العربى:

بعد سرطان الكبد من أكثر أنواع السرطانات التى تؤدى الى الوفاة فى شتى أنحاء العالم. لذلك وضعت العديد من الإستراتيجيات لتحسين تلك المضاعفات، حيث أن الإكتشاف والمعالجات المبكرة والوقاية تلعب دورا حيويا فى إختزال عدد السكان المصابين بهذا المرض. لذلك أجريت الدراسة الحالية بهدف إستبيان مدى فعالية خمسة من المستحضرات الميثانولية للأغذية الوظيفية ذات الأصل النباتى وهى الحبة السوداء، حبوب الشعير، حبوب الشوفان، الكركم وكذلك الصمغ العربى ومخلوطها المضافة الى الخبز التوستلمعالجة أضرار الكبد فى الفئران المستحث برابع كلوريد الكربون. ولقد أوضحت النتائج أن معاملة الفئران برابع كلوريد الكربون قد تسبب فى زيادة معنوية ($p \leq 0.05$) فى نشاط انزيمات الكبد ALT, AST, ALP وذلك بنسب ١٤٢,٧٢ ، ٢٣٨,٢١، ٣٧,٠٢ % مقارنة بالمجموعة الضابطة الطبيعية. كما أدى خلط خبز التوست بالمستحضرات الميثانولية للأغذية الوظيفية مثل الحبة السوداء، حبوب الشعير، حبوب الشوفان، الكركم وكذلك الصمغ العربى ومخلوطها المضافة بنسبة ٠,١% (وزن/وزن) ومخلوط منها بنسب متساوية قد أحدث إنخفاضا معنويا ($p \leq 0.05$) فى درجة نشاط تلك الإنزيمات وبمعدلات مختلفة طبقا لنوع الغذاء المخلوط. ولقد سجل نفس السلوك لمستوى مركب المالونالدهيد فى كرات الدم الحمراء والذى يعد مؤشرا حيويا على الجهد التأكسدى والإلتهابات فى أنسجة الجسم المختلفة. ولعل نتائج الدراسة الحالية تؤيد الفرضية أن مستخلصات النباتات الوظيفية تحتوى على العديد من مجموعات المركبات النباتية الطبيعية بجانب مركبات أخرى يكون لها القدرة على منع أو تثبيت السمية الكبدية لرابع كلوريد الكربون والتى تشمل حفض النشاط لإنزيمات الكبد، ونقص تكوين المالونالدهيد فى كرات الدم الحمراء. لذلك توصى الدراسة الحالية بخلط مستخلصات الأغذية الوظيفية بتركيز ٠,١% فى الأطباق والمشروبات اليومية وكذلك كإضافات أو مدعمات غذائية

الكلمات المفتاحية: الحبة السوداء، حبوب الشعير، حبوب الشوفان، الكركم، الصمغ العربى، وظائف الكبد، الصفراء، المالونادهيد،