Benzo(*a*)pyrene induced liver disorders in rats: possible protective effects of mulberry (*Morus alba* L.) leaves

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اضطرابات الكبد التي يسببها البنزوبيرين في الفئران: الآثار الوقائية المحتملة لأوراق التوت (Morus alba L.)

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هدفت الدراسة الحالية إلى معرفة التأثيرات الوقائية المحتملة لأوراق التوت على إصابات كبد الفئران التي يسببها مركب البنزوبيرين. لذلك تم تحضير مسحوق أوراق التوت (MLP) وتم تحديد التركيب الكيميائى، ومحتوى المركبات النشطة بيولوجيا ، والنشاط المضادات للأكسدة. ولقد أشارت النتائج التي تم الحصول عليها إلى أن MLP يحتوي على مستويات عالية من البروتين والرماد والألياف الخام والكربوهيدرات والفيتامينات المضادة للأكسدة (A ، C ،E) ومضادات الأكسدة غير المغذية (الفينولات والكاروتينات) إضافة الى النشاطً العالى المضاد للأكسدة (65.54٪). أما بالنسبة للتجارب البيولوجية ، تمت تغذية MLP بتركيزات تتراوح من 1 إلى 4 ٪ في النظام الغذائي الأساسي للفئران لمدة أسبوعين ثم حقنها بالبنزوبيرين للحث على إصابات الكبد. تسبب معاملة الفئران بمركب البنزوبيرين الى حدوث زيادة معنوية ($p\leq0.05$) فى مستوى نشاط الإنزيمات المعبرة عن وظائف الكبد (69.77 ٪AST (، 99.19) ٪ALT (، 131.93) ٪ALP (مقارنة بالمجموعة العادية. أيضًا ، أدت معاملت الفئران بمركب البنزوبيرين إلى حدوث زيادة معنوية ($p\leq0.05$) في نشاط إنزيمات استقلاب الدواء (السيتوكروم P450 ، Cyt P450) وانخفاض معنوي ($p\leq0.01$) في كل من مسنوى الدهون الثلاثية في الدم ومحتوى الجليكوجين في الكبد وذلك بنسبة 47.53 ، – 71.80 ، –77.89٪ على التوالي. كما أدت تزويد الوجبات الغذائية للفئران بـ MLP الى حدوث منعا جزئيًا لارتفاع أنشطة إنزيمات وظائف الكبد (AST – ALT – ALP) ، Cyt P450 وكذلك انخفاض مستوى الدهون الثلاثية في الدم ومستوى الجليكوجين في الكبد. تشير نتائج هذه الدراسة إلى أن التغذية على الـ MLP أثبت أنه مفيد في معالجة إصابات الكبد التي يسببها مركب البنزوبيرين. لذلك ، توصى نتائج هذه الدراسة بتضمين الـ MLP بنسبة تبلغ حوالي 4٪ في وجباتنا الغذائية اليومية والمشروبات والمنتجات الغذائية.

الكلمات المفتاحية: مسحوق أوراق التوت، التركيب الكيميائي، الجليكوجين، انزيم السيتوكروم ب450 ، الدهون الثلاثية.

Benzo(a)pyrene induced liver disorders in rats: possible protective effects of mulberry (Morus alba L.) leaves

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Abstract: The present study aimed to investigate the potential protective effects of mulberry leaves on rat liver injuries induced by Benzo(a)pyrene [B(a)P]. Mulberry leaves powder (MLP) was prepared and its chemical composition, bioactive compounds content and antioxidant activity were detected. The obtained data indicated that MLP contain high levels of protein, ash, crude fiber, carbohydrates, antioxidant vitamins (A, C and E) and non-nutrient antioxidants (phenolics and carotenoids) as well as exhibited high antioxidant activity (65.54%). For biological experiments, MLP has been fed at concentrations ranged 1 to 4 % in basal diet of rats for 2 weeks then injected with B(a)P to induce liver injuries. Treatment of animals with B(a)P caused a significant increased ($p \leq 0.05$) in AST (69.77%), ALT (99.19 %) and ALP (131.93 %) compared to normal group. Also, B(a)P treatment brought a significant ($p \leq 0.05$) increase in the activity of drug metabolizing enzymes (cytochrome P450, Cyt P450) and significant ($p \leq 0.01$) decrease in both serum triglycerides and liver glycogen content by the ratio of 47.53, -71.80 and -77.89%, respectively. Supplementation of the rat diets with MLP prevented partially the rise of liver function enzymes activities (AST, ALT and ALP) and cytochrome P450 (Cyt P450) as well as decrease in both serum triglycerides and the liver glycogen level. The results of this study suggest that treatment with MLP proved beneficial on manipulation of the liver injuries induced by B(a)P. Therefore, we recommended MLP by a concentration of about 4% to be included in our daily diets, drinks and food products.

Keywords: Mulberry leaves powder, chemical composition, glycogen, cytochrome P450, triglycerides.

Introduction

Liver is the largest solid organ/gland in the human body. It plays major several vital functions including bile production, absorbing and metabolizing bilirubin, supporting blood clots, metabolizing carbohydrates and fats, vitamin and mineral storage, helps metabolize proteins, filters the blood, immunological function, helps metabolize proteins, production of albumin, and synthesis of angiotensinogen (Voet and Voet, 1990). Also, liver plays a very important part in biotransformation and removing the xenobiotic from the body (Crawford, 1999; Elhassaneen, 1996 and Kebamo, et al., 2015). The enormous functional reserve of the liver often masks the clinical impact of early liver damage. With progression of diffuse disease or disruption of bile flow, however, the consequences of liver damage can easily become life-threatening. Therefore, liver diseases are a major problem throughout the world (Lawrence and Emmet, 2012). The burden of liver diseases has been increasing in Egypt with a doubling of the incidence rate in the past 10 years. This has been attributed to several biological e.g. virus infection and environmental/dietary factors e.g. pesticides, aflatoxin, polycyclic aromatic hydrocarbons (Lorenz, 1985; Elhassaneen, 2004; Elhassaneen, et al., 2016 and Jors, et al., 2018). Other factors such as cigarette smoking, occupational exposure to chemicals such as and heavy metals, and endemic infections in the community, as schistosomiasis, may have additional roles in the etiology or progression of the disease (Anwar, et al., 2008 and Kenko, et al., 2017). The World Health Organization (WHO, 2010) reported that Egypt has one of the highest incidences of hepatitis C, one of the main causes of liver cancer, in the world. The number of deaths resulting from liver cancer in Egypt had risen from 4% in 1993 to 11% in 2009.

It has been known that cooking/processing can produce toxic compounds in foods, if the appropriate precursors are present (Gray and Morton, 1981; Elhassaneen and Tewfik, 1998, and Elhassaneen and El-Badawy, 2013). Amongst of these compounds, polycyclic aromatic hydrocarbons (PAH) from incomplete combustion occur in several foods such as charcoal broiled, roasting, frying and smoked goods (Emerole, et al., 1982;

Larsson, et al., 1983; Van Maanen, et al., 1994 and Bassiouny, 1999). B(a)P is a member of the family, polycyclic aromatic hydrocarbon (PAH) that is a by-product of incomplete combustion or burning of organic (carbon-containing) items, e.g., cigarettes, gasoline, and wood. B(a)P is commonly found with other PAHs in cigarette smoke, in fried, grilled and broiled foods, and as a by-product of many industrial processes (Elhassaneen, 2004; Elhassaneen and El-Badawy, 2013; and Pokhariyal et al., 2019). B(a)P is also found in ambient (outdoor) air, indoor air, and in some water sources (U.S. Environmental Protection Agency, 2005). Many of PAH compounds including B(a)P have been shown to be toxic, mutagenic and/or carcinogenic by extensive experiments in vivo (Harvey, 1985; Plakunov, et al., 1987 and Hawkins, et al., 1990) and in vitro (Elhassaneen, 1996 and Elhassaneen, 2002) systems. Also, B(a)P exposure is associated with the development of liver toxicity and carcinogenicity in all vertebrata (Harvey, 1985 and Hawkins, et al., 1988 and 1990; Elhassaneen, 1996 and Elhassaneen, 2002). It is known that the toxic, tumorigenic and carcinogenic effects of B(a)P correlate with the cellular metabolism of this compounds to arene oxides, phenols, quinones, dihydrodiols, and epoxides and with their subsequent formation of reactive intermediates that interact covalently with DNA to form adducts (Harvey, 1985; Elhassaneen, 1996, Kaarthik, et al., 2012 and Cao, et al., (2020). While the Fixation of a biochemical changes by cell proliferation is considered the next step. The mutagenicity of B(a)P is dependent upon metabolic activation. So, B(a)P is considered a promutagen (Elhassaneen, 1996).

Liver recent pharmacological therapy is costly and associated with several side effects resulting in patient non-compliance. Thus, there is a need to explore alternative therapies particularly from herbal/plant sources as these are cost effective and possess minimal side effects. Also, it was reported that some plant parts exercise various bioactivities, including antioxidant, anti-inflammatory, antidiabetic, anticarcinogenic, antimutagenic etc (El-Safty, 2012; Elhassaneen and Sayed, 2015; Elmaadawy, 2016; and Aly, et al., 2017). One of the less studied plant is the mulberry (Morus alba L.). Different parts of the mulberry plant have been

used over the centuries in traditional medicine as a common agent to treat a variety of conditions including diabetes, cardiovascular diseases, cancer and for activating the immune system through potent antioxidant activity (Butt et al., 2008). The mulberry leaves are nutritious, palatable and nontoxic (Srivastava, et al., 2003). Several studies indicated that mulberry leaves contain many nutrients such proteins, dietary fiber and carbohydrates; minerals such iron, zinc, calcium, magnesium and phosphorous and vitamins such A, B, C, D and E (Ewa, et al., 2013 and Anastasia-Varvara and Fotini , 2016). Also, many bioactive compounds such flavonoids, phenolics acids, quercetin, isoquercetin and alkaloids have been found in mulberry leaves (Doi, et al., 2001, and Anastasia-Varvara and Fotini , 2016). Such bioactive compounds found in mulberry leaves possesses medical benefits, including diuretic, hypoglycemic, antibacterial, antiviral, hypotensive properties and neuroprotective functions (Harauma, et al., 2007). Unfortunately, there is a dearth of information regarding the effect of mulberry leaves on liver diseases. Therefore, the present aims to investigate the potential protective effects of mulberry leaves on rat liver injuries/disorders initiated by Benzo(a)pyrene.

Materials and Methods

Materials

Mulberry leaves were obtained from Benha Center villages, Benha Governorate, Egypt. Benzo(a)pyrene was purchased from Sigma Chemical Co. (St. Louis, MO, Company agent, Cairo, Egypt). Casein was obtained from Morgan Chemical Co., Cairo, Egypt. All organic solvents, buffers and other chemicals of analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Preparation of mulberry leaves powder (MLP)

Mulberry leaves were washed and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., PA) at two stages 50°C for 5 h followed by 40°C for 10 h until arriving by the moisture in the final product to about 7%. The dried peels were ground into a fine powder in high mixer speed (Moulinex

Egypt, Al-Araby Co., Benha, Egypt). The dried powder passed through an 80 mesh sieve the retained powder was used.

Determination of proximate chemical composition, bioactive compounds and antioxidant activities of MLP

MLP samples were chemically analyzed for moisture, protein, fat, ash, fiber and essential oil contents were determined using the methods described in the A.O.A.C. (1995). Carbohydrates calculated by differences using the following equation: carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

Total phenolics and carotenoids in MLP samples were analyzed as follow: MLP was extracted with 80% acetone and centrifuged at 10,000g for 15 min. The supernatant obtained from samples were used for the analysis of total phenolics, carotenoids, and antioxidant activity (AA). Total phenolics were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). The total carotenoids in 80% acetone extract were determined by using the method reported by Litchenthaler, (1987).

Vitamins (A, C, and E) were extracted according to the methods previously described by Epler, et al., (1993); Moeslinger, et al., (1994) and Hung , et al., (1980) and analyzed by HPLC techniques, respectively. Under the chromatographic conditions used, mean values ±SD of vitamins A, C and E recoveries were 89.56± 2.56, 90.65 ±2.54 and 86.56±1.98%, respectively.

Antioxidant activity of MLP extracts and standards (α-tocopherol, BHA, and BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the β-carotene bleaching method following a modification of the procedure described by Marco (1968).

Biological Experiments

Animals

Animals used in this study, adult male albino rats (170±8.5 g per each) were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Basal Diet

The basal diet was prepared according to the following formula as mentioned by (AIN, 1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamins and salts mixture component were that recommended by Campbell, (1963) and Hegsted, (1941), respectively.

Experimental design

All biological experiments performed a complied with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council (NRC, 1996). Rats , n=36 rats and weighted 170±8.5 g per each, were housed individually in wire cages in a room maintained at 25 ± 5 ^{0}C, relative humidity (51±5%), a 12-hr lighting cycle 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 the experimental groups. The first group (Group 1, 6 rats, as a negative control group) still fed on basal diet (BD) and injected with NaCI solution containing 0.1% Tween 20 (5 ml/kg body weight) which was used as a vehicle for the treatment of animals in B(a)P group. Animals in group (2) was challenged with an ip injection of B(a)P (100 mg/5 ml/kg body weight) dissolved in 0.9% NaCI solution containing 0.1% Tween 20 to induce liver impaired rats according to the methods of Hasegawa, et al., (1995). Groups (3-6) rats were classified and feeding BD containing 1, 2, 3 and 4% (w/w) MLP, respectively. The treatment with MLP to the animal belonging to groups (3) to (6) was started 14 days prior to B(a)B treated. All the rats had free access to the diet and water as well as the treatments continued for a total duration of 8 weeks.

Blood sampling

At the end of the experiment, which lasted for eight weeks, blood samples were collected after 12 hours of fasting of rats using the abdominal aorta, after the rats were scratched under the influence of anesthetic ether. Blood samples were received in

clean, dry centrifuge tubes and allowed to clot at room temperature, then were treated with a centrifuge for ten minutes at 3000 rpm for serum separation according to Drury and Wellington, (1980). Serum was carefully withdrawn, transferred to clean tubes and stored frozen at -20 ° C until analysis.

Hematological analysis

Liver functions

Serum aspartate aminotransferase (AST) and Serum alanine aminotransferase (ALT), and Serum alkaline phosphatase (ALP) activities were measured in serum using the modified kinetic method of Tietz, et al., (1976) and Vassault, et al., (1999), respectively.

Liver glycogen level

Liver glycogen levels were determined after digestion of liver and precipitation of glycogen by Glycogen Assay Kit II (Colorimetric, abcam kits Co., ab169558, www.abcam.com).

Serum triglycerides

Enzymatic determination of triglycerides in serum was conducted according to the method of Fossati and Precipe, (1982).

Drug Metabolizing Enzymes (Cytochrome P-450)

Cytochrome P-450 was measured by the carbon monoxide difference spectrophotometry of dithionite-reduced samples by using the method of Omura and Sato ,(1964).

Statistical Analysis

All measurements were done in triplicate and recorded as mean±SD. Statistical analysis was performed with the Student t-test and MINITAB 12 computer program (Minitab Inc., State College, PA). Data were expressed as mean ± SD. The significance of differences was determined by one-way ANOVA followed by Duncan's test for multiple comparisons using a MINITAB 12 computer program. A probability level of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Proximate chemical composition, bioactive compounds, essential oil value and antioxidant activities of MLP

Proximate chemical composition, bioactive compounds, essential oil value and antioxidant activities of MLP are shown in Table (1). The results showed that the moisture content was 7.45%, total protein was 16.22 %, crude fat was 1.98 %, crude fiber was 9.56 %, ash content was 2.95% and total carbohydrate content was 61.84 %. Also, the total energy was recorded 330.06 Kcal/100g. The proximate composition reported was accordance with that observed by Butt ,et al., (2008), Ewa ,et al., 2013 and Anastasia-Varvara and Fotini , (2016). Also, through the review of many studies, it became clear that the chemical and nutritional composition of mulberry leaves is affected by the surrounding environment such as soil, climatic conditions and others (Małgorzata, 2015 and Anastasia-Varvara and Fotini , 2016). All of these components in MLP might be important from the nutrition point of view. Therefore, enrichment of different food products, diets and dishes with MLP would enhance their nutritional quality. On the other side, antioxidant activity and bioactive compounds of MLP were illustrated in the same Table. From such data it could be noticed that, MLP exhibited high level of antioxidant activity (AA, 65.54%). Such high level of antioxidant activity ould be attributed to high content of the measured bioactive compounds such antioxidant vitamins and phytochemicals. The content of antioxidant vitamins were recorded 9.31, 189.15 and 4.11 mg/100g of vitamins A, C and E, respectively. The content of total phenolic compounds and total carotenoids were recorded 91.05 mg GAE.g-^{1} and 59.91 mg/100g, respectively. Such antioxidants nutrient such as vitamins C, E and β-carotene for which there are Dietary Reference Values (DRVs) were recorded in MLP. However, there are thousands of other bioactive compounds in foods that have antioxidant activity but are not classified as "nutrients." These "non-nutrient antioxidants" include phenolics and carotenoids found in MLP (Ajila, et al., 2008). Data of the present study are in accordance with that

observed by Ewa, et al., (2013) and Anastasia-Varvara and Fotini, (2016). Also, numerous studies indicate significance of the antioxidative properties of the white mulberry in preventing and treating lifestyles diseases (Małgorzata, 2015 and Sanchez-Salcedo, et al., 2015). In this direction, many studies indicated that there was a positive and significant ($p \leq 0.01$) relationship between all of the MLP measured bioactive compounds and the antioxidant activity in different plant parts (Khoneem, 2009; Jaggi, 2012 and Elhassaneen, et al., 2013; and Aly, et al., 2017). Antioxidants help protect cells from the potentially damaging physiological process known as oxidative stress. It is thought that oxidative stress be associated with the development of chronic diseases including cancer, heart disease, diabetes, rheumatoid arthritis, obesity, conditions of ageing including neurodegenerative diseases such as Parkinson's and Alzheimer's disease (Chaitanya , et al., 2010 and Elmaadawy, et al., 2016; Aly, et al., 2017 and El-Harbi, 2018). Such phytochemical composition and antioxidant activities properties of MLP are giving impression "such food could be used successfully as functional food".

Table 1. Proximate chemical composition, bioactive compounds, essential oil value and antioxidant activities of MLP

Component	Content
Proximate chemical composition:	
Water (g/100g)	7.45 ± 0.91
Total protein (g/100g)	16.22 ± 2.11
Crude fat (g/100g)	1.98 ± 0.43
Ash (g/100g)	2.95 ± 1.10
Crude fiber (g/100g)	9.56 ± 1.65
Carbohydrate (g/100g)	61.84 ± 4.02
Total energy (Kcal/100g)	330.06 ±10.45
Bioactive compounds:	
Essential oil (g/100g)	1.01± 0.24
Vitamin A (mg/100g)	9.31 ± 1.45
Vitamin C (Ascorbic acid, mg/100g)	189.15 ± 12.50
Vitamin E (mg/100g)	4.11 ± 0.76
Total carotenoids (mg/100g)	59.91 ± 6.03
Total phenolics content (mg GAE.g-^{1}) methanol extract	91.05 ± 3.87
Antioxidant activity:	
Antioxidant activity (AA, %) - aqueous extract	65.54 ± 4.10

Each value represents the mean of three replicates ±SD.

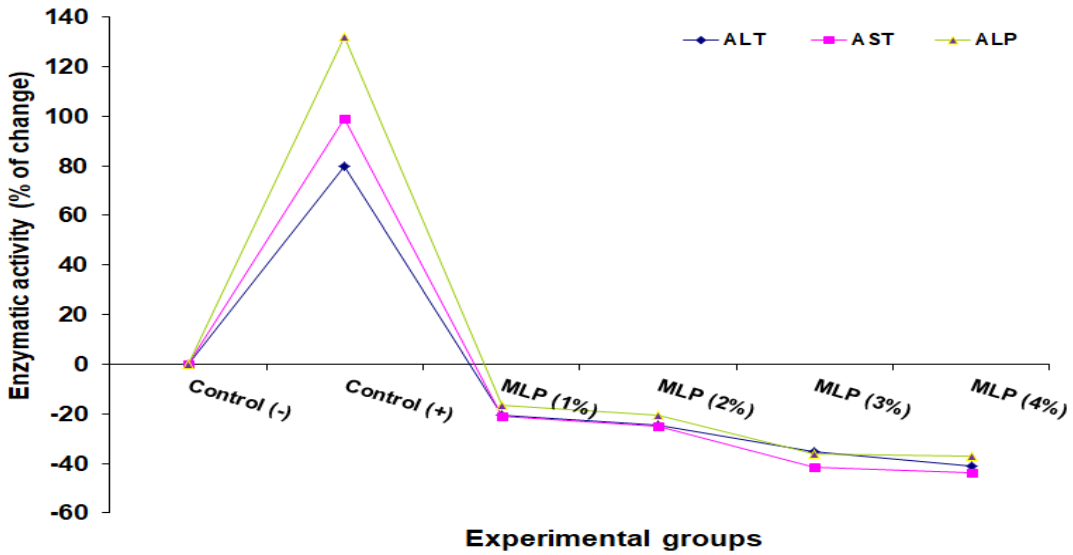
Effects of MLP on liver functions disorders of rats induced by B(a)P

Liver functions of rats injected B(a)P and consumed MLP were shown in Table (2) and Figure (1). From such data it could be noticed that treatment of animals with B(a)P caused a significant ($p \leq 0.05$) increased in ALT (99.19%), AST (79.77%) and ALP (131.93%) compared to normal controls. Supplementation of the rat diets with MLP (1-4% w/w) prevented the rise of mean serum activities of ALT, AST and ALP. The rate of prevention was increased with the increasing of the MLP supplementation ratio. Liver blood tests (ALT, AST and ALP) are some of the most commonly performed blood tests. Such tests can be used successively to assess liver functions or liver injury. An initial step in detecting liver damage is a simple blood test to determine the level of certain liver enzymes (proteins) in the blood. Under normal conditions, such enzymes mostly reside within the cells of the liver but when the liver is injured for any reason such enzymes are spilled into the blood stream. Among the most sensitive and widely used liver enzymes are the aminotransferases. They include aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Such enzymes are normally predominantly contained in liver cells. If the liver is injured, the liver cells spill such enzymes into the blood stream and raising their enzyme blood levels subsequently signaling liver disease.

Table 2. Effects of MLP on liver functions disorders of rats induced by B(a)P

Value	Control (-)	Control (+)	MLP (%, w/w)			
			1	2	3	4
Serum alanine aminotransferase (ALT,U/L)						
Mean	43.12 [d]	85.89 [a]	67.76 [b]	64.36 [b]	50.18 [c]	48.45 [c]
SD	6.32	11.46	10.94	7.12	7.22	11.48
% of Change	0.00	99.19	-21.11	-25.07	-41.58	-43.59
Serum aspartate aminotransferase (AST,U/L)						
Mean	68.20 [d]	122.60 [a]	97.67 [b]	92.45 [b]	79.56 [c]	72.52 [d]
SD	6.98	5.83	10.36	7.05	9.85	12.43
% of Change	0.00	79.77	-20.33	-24.59	-35.11	-40.85
Serum alkaline phosphatase (ALP,U/L)						
Mean	152.20 [e]	352.99 [a]	294.90 [b]	281.20 [c]	224.87 [d]	221.45 [d]
SD	13.65	41.00	10.09	30.69	19.96	10.88
% of Change	0.00	131.93	-16.46	-20.34	-36.30	-37.26

* Means in the same row with different letters are significantly different at $p \leq 0.05$.



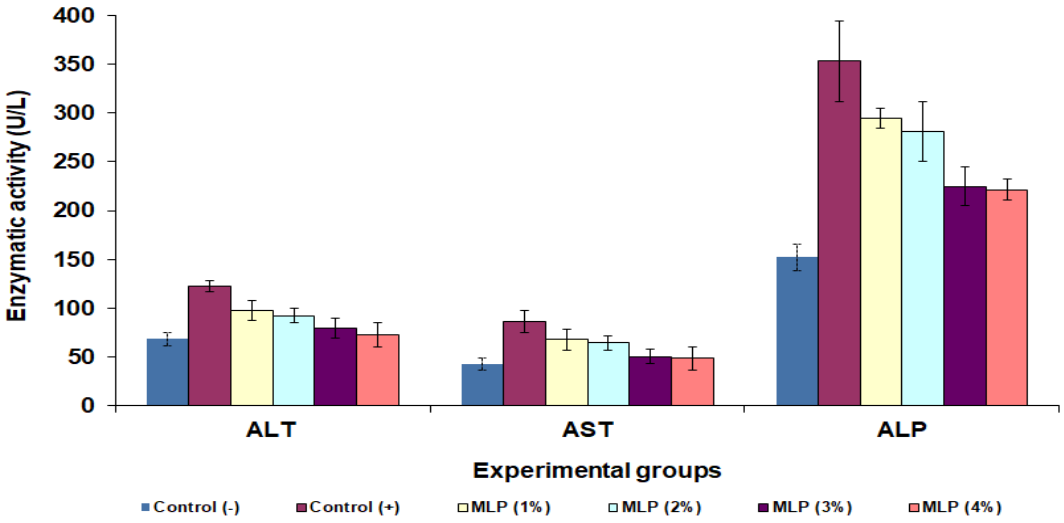


Figure 2. Effects of MLP on liver functions disorders of rats induced by B(a)P

In general, B(a)P was commonly used as a hepatotoxin in the experimental study of liver diseases. The hepatotoxic effects of B(a)P are largely due to the binding of its activated metabolites with the cellular macromolecules and induce peroxidative degradation of membrane lipids of cell wall membrane, mitochondria and lysosomes rich in polyunsaturated fatty acids (Elhassaneen, 1996). Such degradation of cellular membranes is one of the principle causes of hepatotoxicity of B(a)P (Elhassaneen, 2004 and Saleh, 2016). This is confirmed by the elevation noticed in the serum marker enzymes namely AST, ALT and ALP. In related study, Elhassaneen and Al-Badawy, (2013) reported that elevations in liver functions enzymatic activities including AST, ALT and ALP in human as the result of BP consumption in charcoal broiled meat for four weeks. Data of the present study confirmed that MLP is a rich source of different classes of phytochemicals (e.g. carotenoids and phenolics) and antioxidant vitamins (e.g. vitamins A, C and E). Also, organosulfur compounds, flavonoids and alkaloids have been found in MLP (Doi, et al., 2001 and Sanchez-Salcedo et al., 2015). Several previous studies reported that the effect of many plant parts on decreasing the serum liver function enzymes

activity could be attributed to their high level content of such bioactive compounds (El-Sayed, et al., 2012 and Sayed Ahmed, 2016). The possible mode of action of liver serum enzymes-lowering activity of the MLP could be explained by one or more of the following process. MLP bioactive compounds probably block the hepatocellular uptake of bile acids (Dawson, 1998). It could be improved the antioxidant capacity of the liver (Beattic, et al., 2005). Finally, MLP could be improved the of antioxidant defense systems in both serum and red blood cells. Such mechanisms could be confirmed by the study of chan, et al., (2016) who found that mulberry leaves have cardiovascular and hepatoprotective properties. Also, El-Nashar, (2007) reported that pre-treatment with flavonoids such as found in MLP were not only able to suppress the elevation of AST and ALT but also reduce the damage of hepatocytes in vitro. Furthermore, Hassan (2011) found that pre-treatment with apricot kernel extract rich in phytochemicals including polyphenols such as found in MLP were able to reduce the damage of liver i.e. suppress the elevation of AST, ALT and ALP through the improvement of antioxidant defense system in red blood cells.

Effects of MLP on liver glycogen concentration (mg/g tissue) of rats induced by B(a)P

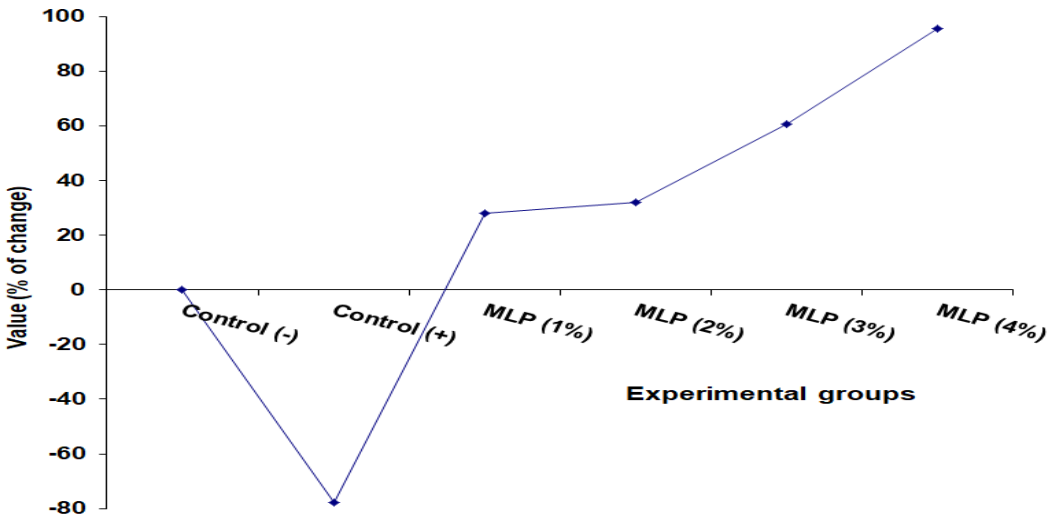
Liver glycogen content of rats injected with B(a)P and consumed MLP were shown in Table (3) and Figures (2). From such data it could be noticed that B(a)P induced significantly ($p \leq 0.05$) decreasing in liver glycogen content by the ratio 77.89%. As the result of MLP consumption, liver glycogen was significantly elevated. The rate of glycogen elevation was increased with the increasing of the MLP concentration. The rate of increasing in glycogen was recorded 28.12, 31.97, 60.87 and 95.87% with the rat diets supplemented by 1, 2, 3 and 4 g/100g of MLP, respectively. Such data are in accordance with the obtained with Fayez (2016) as the result of treatment of liver injury inducing by B(a)P with turmeric and curcumin powders.

In general, glycogen is represents a complex sugar necessary for production of nutrients and energy in the body. Its storage disorders, Glycogen Storage Disease (GSD), are genetic conditions seen in pediatric population (detected at birth in severe types or later in childhood in less severe types) (Christian and Paul, 2011).. They impair the ability of the liver to store and metabolize glycogen. GSD or disorders cause varying degrees of liver enzyme abnormalities. Also, it affects the liver, muscles and other areas of the body. In similar study, Hasegawa et al., (1995) found that previous drinking of green tea clearly protected against the changes in liver glycogen content. Such data with the others suggested that secretion of lipoprotein from liver to blood might be blocked because of intracellular structural failure and/or because of the energy depletion suggested by the marked decrease in glycogen content.

Table 3. Effects of MLP on liver glycogen concentration (mg/g tissue) of rats induced by B(a)P

Value	Control (-)	Control (+)	MLP (%, w/w)			
			1	2	3	4
Mean	14.09^{c}	3.11^{d}	3.99^{c}	4.11^{c}	5.01^{b}	6.10^{a}
SD	1.89	0.17	0.30	0.51	0.77	0.88
% of Change	0.00	-77.89	28.12	31.97	60.87	95.87

* Means in the same row with different letters are significantly different at $p \leq 0.05$



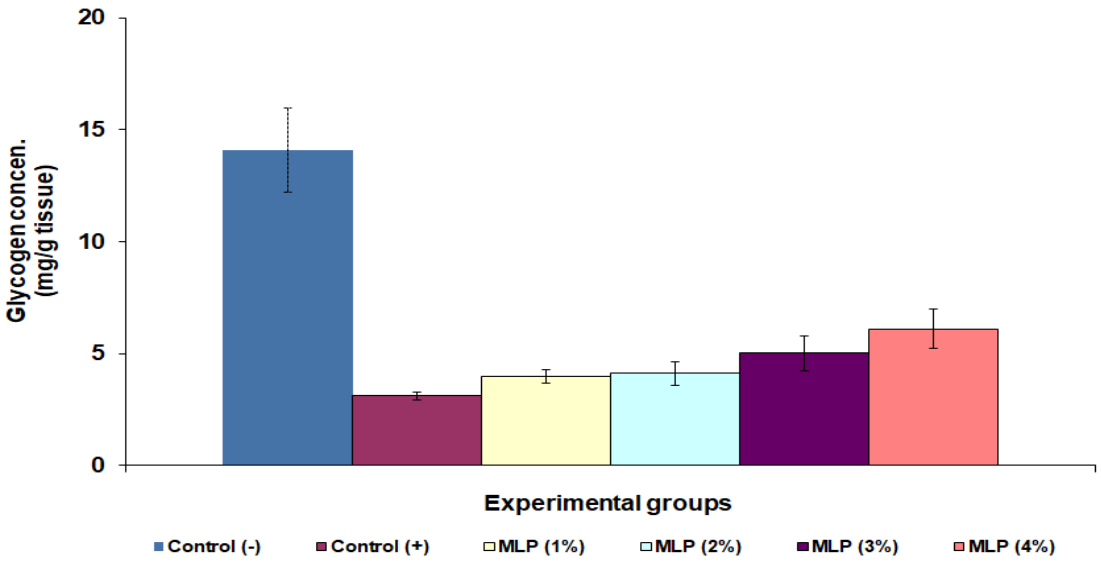


Figure 2. Effects of MLP on liver glycogen concentration of rats induced by B(a)P

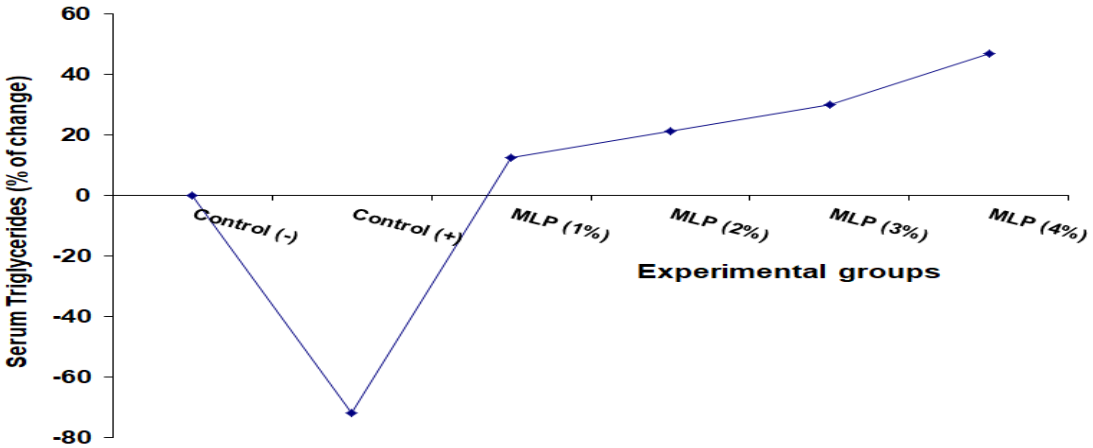
Effects of MLP on serum triglycerides level of rats induced by B(a)P.

Serum triglycerides (TGs) level of rats injected with B[a]P and consumed MLP were shown in Table (4) and Figures (3). From such data it could be noticed that B(a)P induced significantly ($p \leq 0.05$) decreasing in serum TG level by the ratio 71.80%. As the result of MLP consumption, serum TGs was significantly elevated. The rate of serum TG elevation was increased with the increasing of the MLP concentration. The rate of increasing in serum TG was recorded 12.50, 21.22, 30.14 and 47.04% with the rat diets supplemented by 1, 2, 3 and 4 g/100g of MLP, respectively. Such data are in accordance with the obtained with Fayez (2016) as the result of treatment of liver injury inducing by B(a)P with turmeric and curcumin powders.

Table 4. Effects of MLP on serum triglycerides level (mg/dL) of rats induced by B(a)P

Value	Control (-)	Control (+)	MLP (%, w/w)			
			1	2	3	4
Mean	139.10	39.23	44.14	47.56	51.06	57.68
SD	24.16	5.22	4.11	4.40	6.99	7.54
% of Change	0.00	-71.80	12.50	21.22	30.14	47.04

* Means in the same row with different letters are significantly different at $p \leq 0.05$



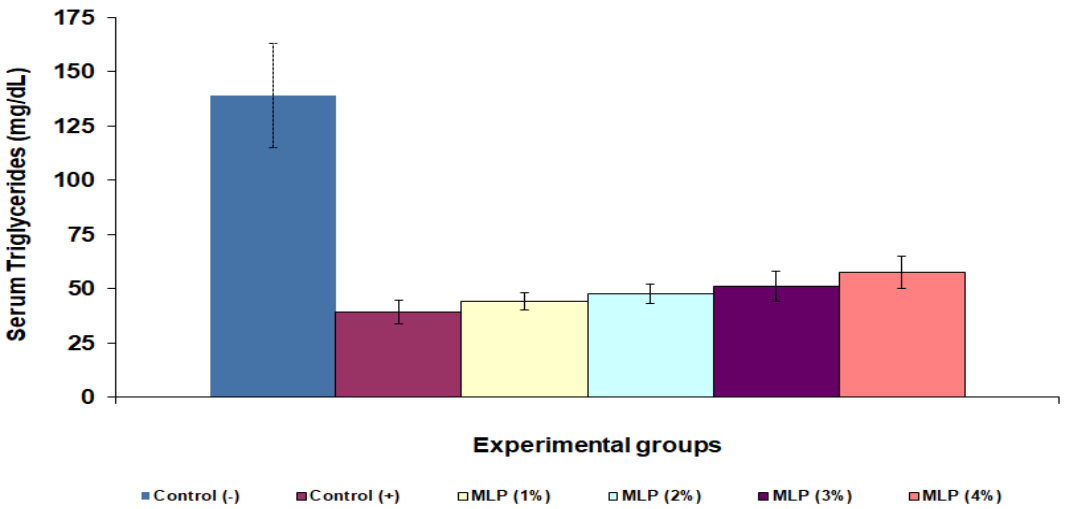


Figure 3. Effects of MLP on serum triglycerides level of rats induced by B(a)P

TGs are a type of fat. They are the most common type of fat in the body. They come from foods and extra calories. When the body needs energy, it releases the triglycerides (voet and Voet, 1990). Very low density lipoprotein (VLDL) cholesterol particles carry the triglycerides to the tissues. Having a high level of triglycerides can raise your risk of heart diseases, such as coronary artery disease. High blood TG levels can be genetic, or caused by diabetes, thyroid problems, kidney disease, or some medicines (Karim et al., 2009). In similar study carried out by Hasegawa et al., (1995) found that previous drinking of green tea clearly protected against the changes in serum TGs level. Also, Asai and Miyazawa., (2001) indicated that dietary curcuminoid lowered liver cholesterol and triacylglycerol, and plasma triacylglycerol. Also Chuengsamarn et al., (2014) found that curcumin lowers the atherogenic risks by reducing the insulin resistance, triglyceride, visceral fat and total body fat.

Effects of MLP on the activity of cytochrome P450 in liver of rats induced by B(a)P

Effects of MLP treatments on the activity of cytochrome P450 in liver of rats subjected to BP treatment was shown in Table (5) and Figure (4). From such data it

Table 5. Effects of MLP on serum triglycerides level (nmol/mg protein) of rats induced by B(a)P

Value	Control (-)	Control (+)	MLP (%, w/w)			
			1	2	3	4
Mean	1.62	2.39	2.19	2.09	1.88	1.74
SD	0.30	0.39	0.42	0.23	0.10	0.19
% of Change	0.00	47.53	-8.37	-12.55	-21.34	-27.20

* Means in the same row with different letters are significantly different at $p \leq 0.05$

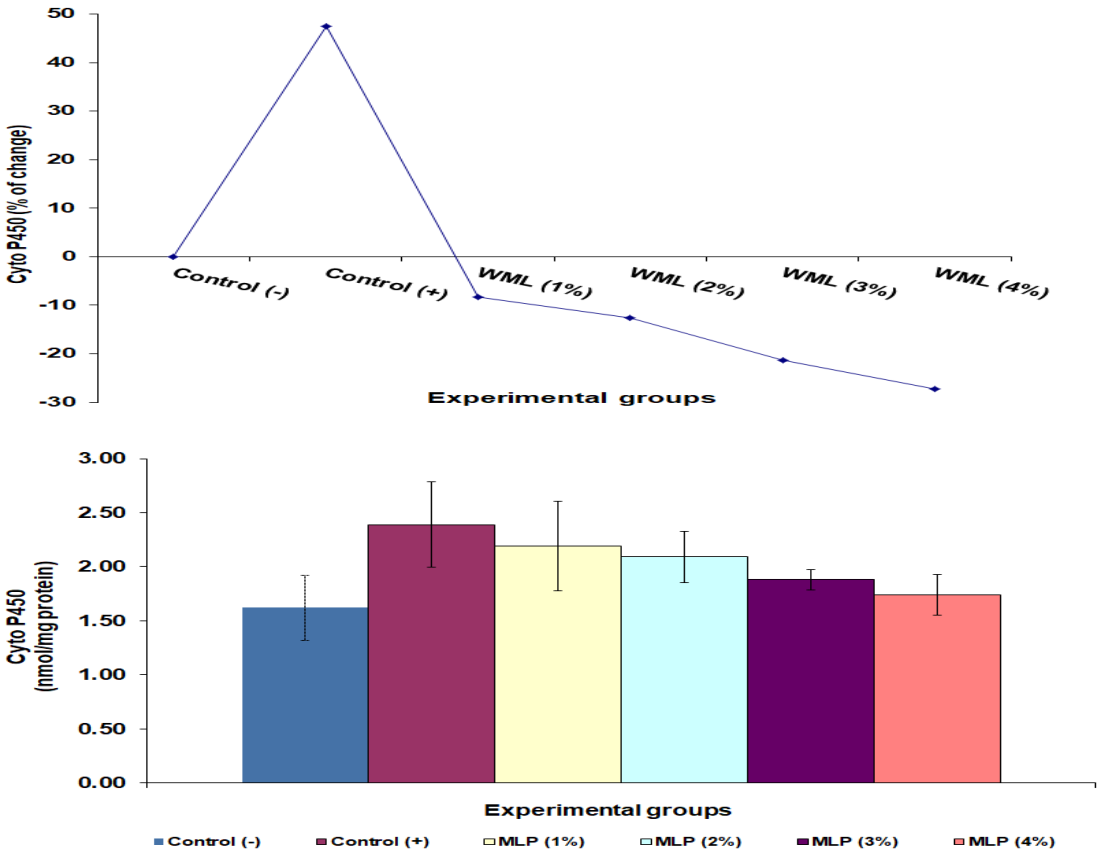


Figure 4. Effects of MLP on the activity of cytochrome P450 in liver of rats induced by B(a)P

could be noticed that treatment of animals with BP caused a significant increased ($p \leq 0.05$) in cytochrome P450 (47.53%) compared to normal control animals. Feeding of the rat diets with MLS (1-4 g/100g w/w) prevented the rise of mean serum cytochrome P450 activity. The rate of preventative was increased with the increasing of the MLS concentration. The rate of decreasing in the cytochrome P450 activities were -8.37, -12.55, -21.34 and -27.20 % with the rat diets blended with 1, 2, 3, and 4 g/100g of MLS, respectively. In similar studies, Liu, et al., (2015) and Cao, et al., (2020) reported that BP treatment brought about a significant increase in the activities of drug metabolizing enzymes (cytochrome P450 and b5) in lungs of mice and the activities of these enzymes were markedly decreased by the administration of phytochemicals including curcumin and phenolic compounds.

Conclusion

B(a)P is considered as a ubiquitous environmental and food contaminants as well as a top risk factor in the development of liver diseases. Feeding mulberry leaves successfully applied as functional food for containing several classes of bioactive compounds and exhibited antioxidant activity that are able to prevent or inhibit liver injuries induced by chemical toxin i.e. B(a)P. Mulberry leaves exhibit inhibiting effects probably by improving the liver functions and modulating regulators of drug metabolizing enzymes (cytochrome P_{450}) and thereby adversely affecting the injuries process to the benefit of the biological system. We recommended that mulberry leaves by a concentration about 4% (w/w) to be included in our daily dishes and drinks as well as in different food products as a natural food additive.

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