تأثير حشيشة الدينار على تحفيز إنزيمات الكبد المضادة للسموم في فئران الألبينو البالغة

Effect of *Humulus lupulus* on Stimulation of Hepatic Detoxifying Enzymes in Adult Albino Rats

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المستخلص:

هدفت الدراسة الحالية في المقام الأول إلى: التحقيق في تأثير الذئبة الدبوسية في مستويات مختلفة (١.٠، ٢.٥، ٥.٠٪) على الإصابة الكبدية التي يسببها بنزو [أ] بيرين في الجرذان البيضاء. تم وزن ثلاثين من ذكور الفئران البيضاء البالغة (١٥٠ ± ١٠ جم لكل منها) المستخدمة في هذه التحقيقات. تم إطعام جميع الفئران على نظام غذائي أساسي لمدة أسبوع واحد قبل بدء تجربة التأقلم. بعد أسبوع واحد، تم تقسيم الفئران إلى ثلاث مجموعات رئيسية ، وفقًا للمخطط التالي لمدة ٤٢ يومًا متتالية: المجموعة (١): تم التحكم في المجموعة السلبية للتحكم في النظام الغذائي الأساسي. المجموعة (٢): السيطرة على النظام الغذائي القاعدي الإيجابي مع العلاج ب [أ] ف. المجموعة (٣): نظام غذائي مستكمل مكمل بمعالج Humulus lupulus pre-B [a] P، وينقسم إلى ثلاث مجموعات على النحو التالي: (١.٠، ٢.٥، ٥.٠٪). في نهاية التجربة، تم خدش الفئران وجمع عينات الدم للتحليل الكيميائي الحيوي. أظهرت النتائج التي تم الحصول عليها أن مكملات Humulus lupulus لها تأثير كبير في الحد من إصابة الكبد التي يسببها B [a] P، عن طريق تقليل إنزيمات الكبد ALT, AST كما خفضت مستوى البيليروبين. بالإضافة إلى الزيادة الكبيرة في إنزيمات إزالة السموم من الكبد (GSH، الجلوتاثيون، CAT, GPX) لتكون قريبة تقريبًا من المعدل الطبيعي، مع تحسين تركيزات MDA. في الخلاصة: أثبتت هذه الدراسة أن المكملات الغذائية من Humulus Lupulus هي عامل فعال وفعال في علاج الكبد، ولديها عوامل وقائية ضد B P [a] التي يسببها تلف الكبد من خلال تحريض الإنزيمات المزيلة للسموم.

الكلمات المفتاحية: حشيشة الدينار ، إنزيمات الكبد ، إنزيمات الكبد المضادة للسموم

Effect of *Humulus lupulus* on Stimulation of Hepatic Detoxifying Enzymes in Adult Albino Rats

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Abstract:

The present study aimed primarily to: investigate the effect of *Humulus lupulus* in different levels (1.0, 2.5, and 5.0 %) on the hepatic injury that induced by Benzo[a]pyrene in adult albino rats. thirty adult male albino rats were weighing (150 ± 10 gm per each) used for these investigations. All rats were fed on basal diet for one week before starting the experiment for acclimatization. After one week, rats were divided into three main groups, according to the following scheme for Successive 42 days: Group (1): control negative was fed on basal diet. Group (2): control positive fed basal diet with B[a]P treatment. Group (3): received diet supplemented with *Humulus lupulus* pre-B[a]P treatment, and sub-divided into three groups as: (1.0, 2.5, and 5.0%) supplementation. At the end of experiment, rats were scarified and blood samples were collected for biochemical analysis. The obtained results revealed that *Humulus lupulus* supplementation has significant action in reducing liver injury induced by B[a]P, by reduction of liver inzymes AST and ALT also reduced bilirubin level. In addition to significant increase in liver detoxifying enzymes (GSH, glutathione, GPX and CAT) to nearly becoming close to normal range, amd improved MDA concentrations. In Conclusion: This study demonstrated that *Humulus Lupulus* dietary supplements is a potent and efficacious hepatoprotective agent, and have the potential as preventive agents against B[a]P induced liver damage through induction of detoxifying enzymes.

Keywords: **Humulus lupulus, Benzo [a]pyrene , detoxifying enzymes , Hepatoprotective , Glutathione.**

1. Introduction

Recently, researches have been interested in the nutrients of natural foods for their functional role in the medical field, especially in the detoxification process that implicated in xenobiotic alteration and exclude. So far, many vitro and vivo studies have been conducted to prove the importance of natural foods nutrients in the detoxification process of the body (Baer-Dubowska and Szaeferet, 2013). Over all, the studies demonstrated that particular food have the ability to stimulate and modulate the xenobiotic metabolic pathway and then excrete it (Moon et al., 2006). Various whole foods such as cruciferous vegetables (James et al., 2012), berries (Aiyer and Gupta, 2010), soy (Bogacz et al., 2014), garlic (Lii et al., 2006), and spice as well, such as turmeric have been proven to have a useful role in the detoxification process (Hsieh et al., 2014).

In the 12th century, Humulus lupulus is known all over the world as the main material in the beer industry that gives it a characteristic aroma and flavor (Olas et al., 2011). Moreover, the ability to preserve beer and prevent spoilage for its polyphenolic compounds and acyl phloroglucides. In addition, there is a lot of hops constituents with antimicrobial activities such as lupulone and humulone in addition to their various isomers and lupulone (beta-acid) is the most antimicrobial constituent (Natarajan et al., 2008). Hops is also one of the plants which have been used in traditional medicine (Zanoli et al., 2002), and as integral part of the human diet (Olas et al., 2011).

Traditional medicinal has used hops as a medical plant since ancient times, that ncludes treatment of anxiety and insomnia, mild sedative, pain relieve, and for stimulation of the gastric function as combating dyspepsia (Zanoli and Zavatti, 2008). Today the plant of hops known around the world as the raw material for beer production. Hop cones rich in polyphenolic compounds and acyl phloroglucides which considered the main compound for beer preservation, flavoring and aroma characteristic (Zanoli and Zavatti, 2008).

In line with the traditional use of hops and it's phytochemical and pharmacological properties. Our study particularly focused on the effect of hops

on the liver injury and toxicity induced by B[a]P, comparing the results obtained in our laboratory with those indicated in other studies.

Noteworthy,the terms polycyclic aromatic hydrocarbons (PAHs) refers to a large group of organic compounds that comprising only carbon and hydrogen and are consisting of two or more aromatic rings (Armstrong et al., 2004). The ring systems can be present in multiple configurations. PAHs start from semi-volatile molecules to molecules with high boiling points (WHO, 1998). Therefore, PAHs could found in both gas and particle phase of ambient air or in mixtures of both phases. More than 500 various PAHs have been discovered in air, frequently, the emphasis is on measuring benzo[a]pyrene (B[a]P) as a representative of the polycyclic aromatic hydrocarbons family (Boström et al., 2002). The European Union and US Environmental Protection Agency (EPA) priority contaminants included PAHs because of their carcinogenic and mutant properties (Anyakora et al., 2005).

The majority routes of human exposure to PAHs occurs by breathing ambient or indoor air, smoking cigarettes, and breathing smoke from open fireplaces (ACGIH, 2005). Eating food contain PAHs, such as food processing (drying and smoking) and using high temperatures to cook foods (grilling, roasting, and frying) considered major sources that lead to PAHs generation. (Chen and Lin, 2004). Some crops can synthesize PAHs or absorb them through contaminated soil, water or air (such as wheat, beans, and lentils). Moreover, PAHs can reach the water by industrial effluents and marine oil shipping (Ciecierska and Obiedziński, 2013). Further, intake of PAHs may occur from contaminated soil via dermal (skin) exposure and inhalation of PAH vapors (Wang et al., 2012). Workers may occupationally exposed to PAHs as a result of inhaling exhaust vapors (such as mechanics, street vendors, or motor vehicle drivers) in addition to those working in petroleum refining, metalwork, or mining (See et al., 2006).

Benzo[a]pyrene (B[a]P) is a highly toxic PAH present in our environment as a food contaminant and air pollutant, which attacks cellular macromolecules and leads to toxicity and carcinogenicity (Shou et al., 1994). Whereas the liver

plays an important role in protecting the organism from potentially toxic chemical insults, including toxic chemicals (Grunhage et al., 2003). The liver is often regarded as a primary target of BaP because this organ contains many of the enzymes involved in bioactivation of the parent molecule. However, 21% of a single BaP dose is available in the liver within 10 minutes, and high B[a]P levels within 30 minutes (Weyand and Bevan, 1986). There have been many attempts to screen phytochemicals with high inhibitory effects on B[a]P toxicity. Among them, about 500 varieties of flavonoids (Havsteen, 1983).

Noteworthy, most of the chemical in the environment are chemically inert in themselves such as BaP and other PAHs, therefore they need to be activated metabolically by metabolizing enzymes to turn to active metabolites and thus easy to exert their toxic effects and carcinogenicity (Luch and Baird, 2005). Among all body organs, the most metabolizing enzymes that responsible for metabolic activation exist in the liver and then followed by lung, intestinal mucosa, skin and kidneys. The metabolic activation also occur in spleen, nasal tissues, brain, mammary gland, placenta, uterus, hair follicles, platelets, erythrocytes, and leukocytes (Anderson et al., 1989).

In this regard, liver considered the center for drug and xenobiotic metabolism and detoxification by drug metabolizing enzymes (DMEs) (Upadhyay et al., 2008). Despite the widespread of hepatotoxicity, however it is not easy to estimate because of the difficulty of diagnosis and unclear symptoms of exposure to these drugs. The mortality rate of liver damage resulting from drugs reached 10% (Singh et al., 2016).

Metabolic reactions and detoxification pathways are of two phases; phase I and phase II (Dahm and Jones, 1996). Phase I metabolism responsible of alternate the basic structure of drug molecule (oxidation, reduction, or hydrolysis), these actions usually occur first, and promote solubility in water by generating hydroxyl, carboxy, or epoxide functional groups on the main compound. These functional groups in turn facilitate the interaction of phase II (conjugation with glucoronate, sulfate, acetate, or glutathione moietie). In general, conjugation activity lead to motivate water solubility and renal excretion

(Vessey, 1996). Additionally, phase II reactions implicated in inhibit hepatic injury that induced by xenobiotic because most conjugates are biologically inactive. However, the disturbance of phase II procedures cause accumulation of hepatotoxic metabolites of phase I (Lee, 1995).

A wide variety of ethnomedicinal plants, which have been claimed to be hepatoprotective due to its potential and efficacy against drug-induced liver toxicity (Singh et al., 2016). The present study represented an assessment of humulus lupulus nutritional and biochemical effects in rats damaged liver induced by B[a]P shown promising signs in the amelioration of the hepatic system and a potential source for new therapeutic agents that could be used in the prevention of hepatic injuries.

Humulus lupulus has been studied in a significant number of published articles. Starting from the second half of the 20th century, several phytochemical studies were performed to investigate the composition of hop cones and other parts of the plant, leading to the isolation and identification of pharmacologically relevant compounds such as flavanones, chalcones, phloroglucinol derivatives. In line with the traditional use of hops and it's phytochemical and pharmacological properties. The present study aimed to investigate the nutritional and biochemical effect of Humulus lupulus on the hepatic injury that induced by Benzo[a]pyrene in adult albino rats.

2. Materials and Methods:

2.1 Materials:

Benzo[a]pyrene, was optained from Sigma chemical company, Cairo, Egypt. Casein, vitamin mixtures, salt mixtures components, cellulose, choline Cholin chloride and L-Cystine used for rats feeding were obtained from El-Gomhorya Company for Chemicals and Drug Trading, Cairo, Egypt.

2.3 Biological Experiments:

2.3.1 Animals:

Thirty adult male albino Sprague-Dawley albino rats weighing (150 ± 10 gm per each) were purchased from Laboratory Animal Colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

2.3.2. Preparation of rats liver injury induced by B[a]P:

B [a]P was dissolved in 0.2 ml corn oil shortly before injection. Rats were treated with B[a]P by intraperitoneal injection (i.p) at a dose of (125 mg/kg body weight).

2.3.3. Basal Diet:

The basal diet prepared according to the following formula as mentioned by (AIN, 1993) as follow: casein (14%), corn oil (4%), vitamin mixture (1%), mineral mixture (3.5%), choline chloride(0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (72%). The used vitamin mixture component was that recommended by (Campbell, 1963) and found in table (2) while the salt mixture used was formulated according to (Hegested, 1941).

2.3.4 Experimental design:

Thirty adult male albino Sprague-Dawley albino rats weighing (150 ± 10 gm per each) were housed in well-aerated wire cages individually. All rats were kept in a room maintained at 25 ± 2 0C, relative humidity (55±5%), exposed to a 12:12-h light-dark cycle and kept under normal healthy conditions. All rats fed on basal diet for one-week (adaptation period) before starting the experiment. Diet was giving in non-scattering feeding cups to avoid loss or contamination of food, water was provided to the rat by means of glass tubes projecting through the wire cage from an inverted bottle supported to one side of the cage. Rats were provided diet and drinking water ad libitum.

After the adaptation period, the sixty six rats were divided into three main groups, (each with 6 rats) were studied according to the following scheme for 35 days:

Group (1): served as negative control one, which normally fed on basal diet without B[a]P treatment.

Group (2): served as served as positive control one, which fed basal diet with B[a]P treatment.

Group (3): received diet supplemented with *Humulus lupulus* pre-B[a]P treatment, and sub-divided into three groups as:

- **Group 3A- received basal diet supplemented with 1% of *Humulus lupulus* dried cones pre-B[a]P treatment.**
- **Group 3B- received basal diet supplemented with 2.5% of *Humulus lupulus* dried cones pre-B[a]P treatment.**
- **Group 3C- received basal diet supplemented with 5% of Humulus lupulus dried cones pre-B[a]P treatment.**

*** BaP was given as i.p injection as a single dose of (125 mg/kg b.w. in 0.2 ml corn oil) four week after humulus lupulus administered in the aforementioned groups**

2.3.2. Blood sampling:

At the end of experiment period (42 days), after 12 hours fasting blood samples were collected using the portal vein. Rats were scarified under slight diethyl ether anesthesia. Serum was separated from non-heparinized blood samples, which receipt into clean dry centrifuge tubes, blood left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m using the method of Drury and Wallington, (1980). Serum was carefully aspirate, transferred into clean covet tubes and kept frozen at -20^{o} C till analysis of parameters later.

All serum samples were analyzed for determination the following parameters: liver functions by measuring aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), and total bilirubin. Kidney functions by measuring creatinine and uric acid. Hepatic detoxifying enzymes: Malondialdehyde (MDA), Reduced Glutathione (GSH), Glutathione, Glutathione Peroxidase (GPX), and serum Catalase.

At the end, obtained results were expressed as Mean ± SD. Data were evaluated statistically with SPSS package program using one-way analysis of variance (ANOVA). Significant difference between means was estimated at $p<0.05$ according to Snedecor and Cochran, (1986).

3. Results:

Effects on liver functions of rat groups fed Humulus lupulus before benzo[a]pyrene treatment (pre-benzo[a]pyrene treatment) in comparison to control rat groups:

Data on table (1) and figures (1, 2 and 3) show the effect of Humulus lupulus supplementation on liver status indicated by enzyme measurements. It could be noticed that liver enzyme status of normal rat group is running within the normal estimated range. Whereas, control positive group that was treated with B[a]P has significant elevated enzyme levels as 88.34 ± 7.12, 65.22 ± 4.10 U/L and 3.85 ± 0.22 mg/dl for AST, ALT and total Bilirubin respectively. Both supplementation levels of Humulus lupulus (2.5 and 5.0%) have a significant reducing action toward all of measured liver enzymes and all elevated liver enzymes approached the normal ratio. This finding indicated that there is significant difference in actio

Table1: Effects on liver functions of rat groups fed *Humulus lupulus* before benzo[a]pyrene treatment (pre-benzo[a]pyrene treatment) in comparison to control rat groups:

Parameters	Group 1 (-Ve Control Group)	Group ٢ (+Ve Control Group)	Group ٣		
			Group 3A 1%	Group 3B 2.5%	Group 3C 5%
AST (U/L)	20.62 ± 3.51[a]	88.34 ± 7.12[c]	80.41 ± 6.32[c]	58.32 ± 7.72[b]	47.66 ± 6.86[b]
ALT (U/L)	16.42 ± 2.15[a]	65.22 ± 4.10[c]	59.5 ± 4.7[c]	49.5 ± 3.4[b]	44.2 ± 3.3[b]
Total Bilirubin (mg/dl)	[a]١.55 ± 0.4١	3.85 ± 0.22[c]	2.99 ± 0.21[c]	2.42 ± 0.25[b]	2.18 ± 0.32[b]

Results are expressed as Mean ± SD. Values at the same row with different letters are significantly different at P < 0.05

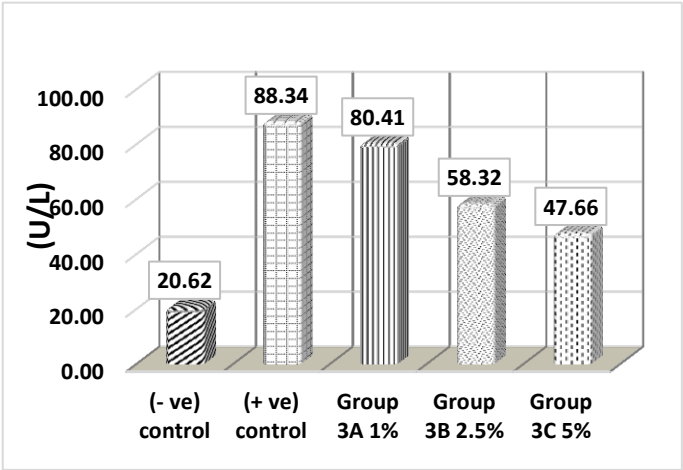

Figure 1: Effect on liver AST of rat groups fed *Humulus lupulus* post-B[a]P treatment in comparison to control rat groups.

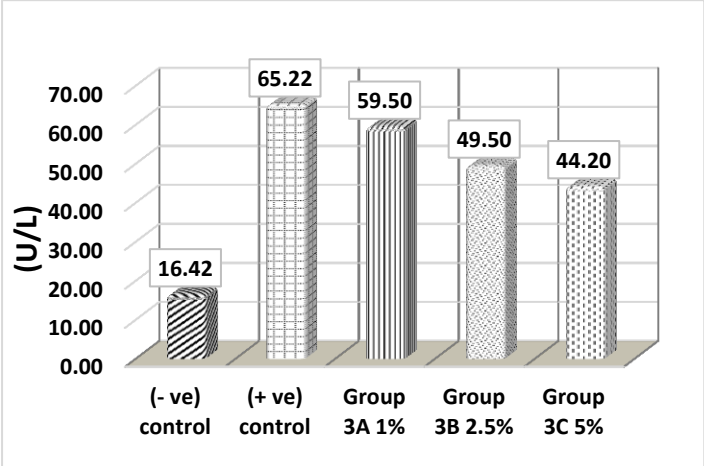

Figure 2: Effect onliver ALT of rat groups fed *Humulus lupulus* post-B[a]P treatment in comparison to control rat groups.

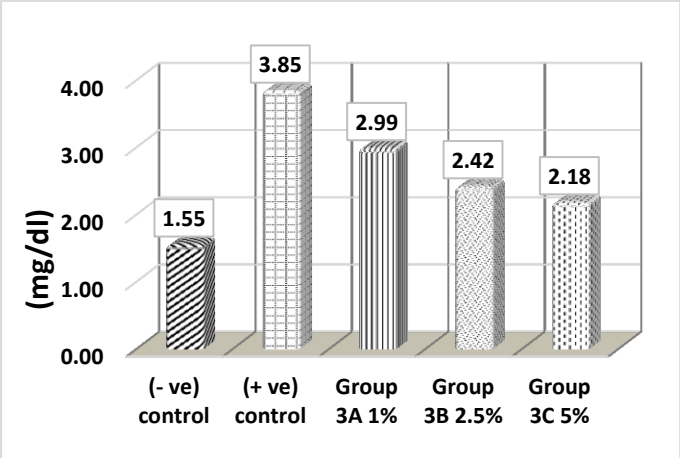

Figure 3: Effect on total bilirubin of rat groups fed *Humulus lupulus* post-B[a]P treatment in comparison to control rat groups.

Effects on detoxifying enzyme titer of rat groups fed *Humulus lupulus* before benzo[a]pyrene treatment (pre-benzo[a]pyrene treatment) in comparison to control rat groups:

Data presented in table (5) and figures (13 and 14) show the effect of *humulus lupulus* supplementation on detoxifying enzyme titre (Glutathione Reduced (GSH), Catalase, malonaldhyde (MDA), and liver glutathione. From these data, it could be observed that normal rat group has the highest production levels of GSH, MDA and liver GSH. Positive control group that was treated with B[a]P has a significant reduction of GSH, MDA and liver GSH production levels as 440.25 ± 21.08 µmol/L, 28.4± 1.1 nmol/L and 2.5.± 0.003 nmol/mg respectively. There is a significant increase in GSH, MDA and liver GSH at the Pre-B[a]P treated groups supplemented with *humulus lupulus*. However, the level of 5.0 % *humulus lupulus* supplemenatation had the highest improving action of GSH, MDA and liver GSH production that are nearly becoming very close to that of normal range, which recorded in normal rat group and the concentrations were 620.30 ± 45.22 µmol/L, 19.6± 4.3 nmol/L and 3.5.± 0.004 nmol/mg respectively. Also, It could be noticed that catalase concentration of normal rat group is running within the normal estimated range as 62.3 ±2.4 nmol/L. Whereas, control positive group that was treated with B[a]P has significant reduced as 33.4 ±1.1 nmol/L. *Humulus lupulus* supplementations have significant improving action for catalase concertation concertation as 51.6 ± 4.3 nmol/L that become nearly to normal level.

Table 2: Effects on detoxifying enzyme titre of rat groups fed *Humulus lupulus* before benzo[a]pyrene treatment (pre-benzo[a]pyrene treatment) in comparison to control rat groups:

Parameters	Group 1 (-Ve Control Group)	٢Group (+Ve Control Group)	٣Group		
			Group 3A 1%	Group 3B 2.5%	Group 3C 5%
Glutathione Reduced (GSH) (µmol/L)	970.33 ± 88.15[a]	440.25 ± 21.08[c]	500.25 ± 12.10[c]	575.20 ± 10.18[c]	620.30 ± 45.22[b]
Catalase (nmol/L)	62.3 ± 2.4[a]	33.4 ± 1.1[c]	39.5 ± 3.3[c]	45.3 ± 3.8[b]	51.6 ± 4.3[b]
MDA (nmol/L)	11.7 ± 0.02[a]	28. 4 ± 0.01[c]	27.5 ± 0.03[c]	24.5 ± 0.01[b]	19.6 ± 0.02[b]
Liver Glutathione (nmol/gm)	5.2 ± 0.002[a]	2.5 ± 0.003[a]	2.8 ± 0.002[a]	3.1 ± 0.0032[a]	3.5 ± 0.004[a]
Glutathione peroxidase (mg/ml)	.23 ± 4.10[a]٤4	25.15 ± 3.23[c]	27.22 ± 2.55[c]	29.15 ± 3.25[c]	32.45 ± 3.55[b]

Results are expressed as Mean ± SD. Values at the same row with different letters are significantly different at P < 0.05

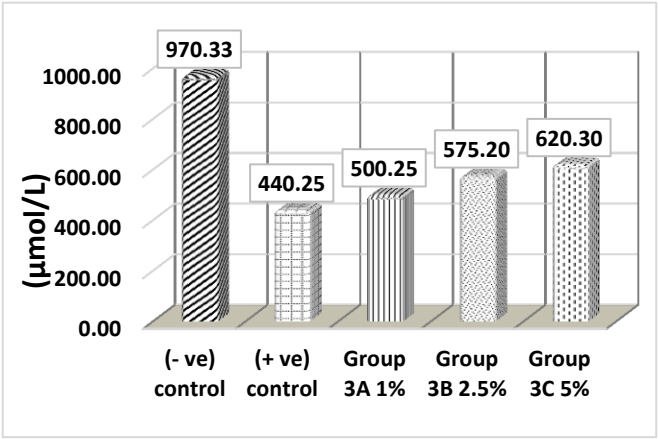

Figure 4: Effect on glutathione reductase of rat groups fed Humulus lupulus post-B[a]P treatment in comparison to control rat groups.

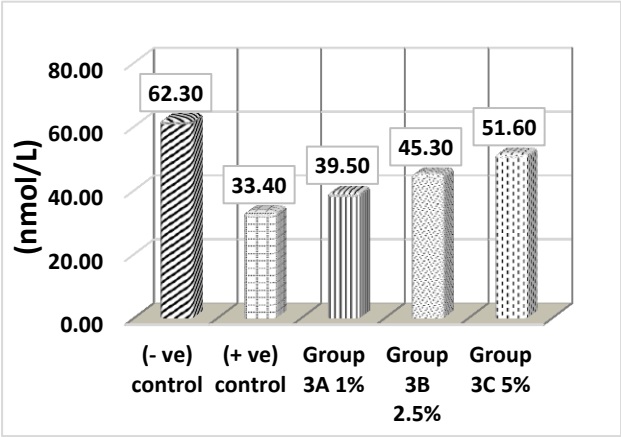

Figure 5: Effect on catalase of rat groups fed Humulus lupulus post-B[a]P treatment in comparison to control rat groups.

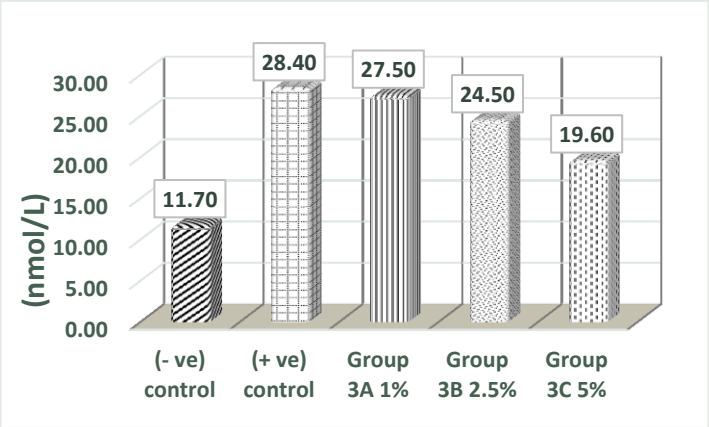

Figure 6: Effect on malonaldhyde of rat groups fed Humulus lupulus post-B[a]P treatment in comparison to control rat groups.

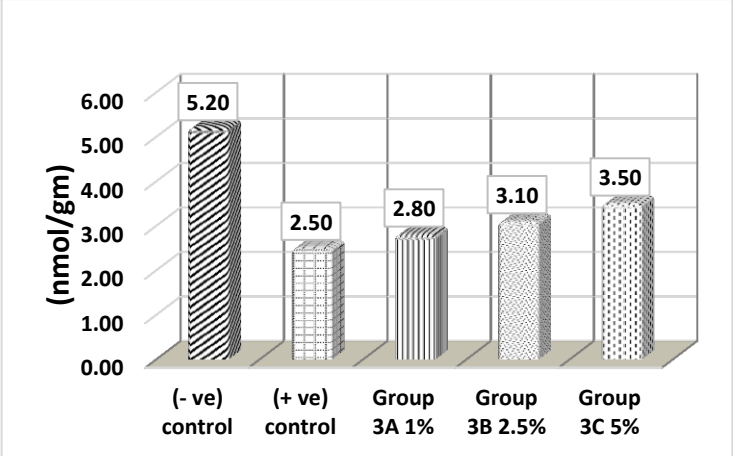

Figure 7: Effect on glutathione of rat groups fed Humulus lupulus post-B[a]P treatment in comparison to control rat groups.

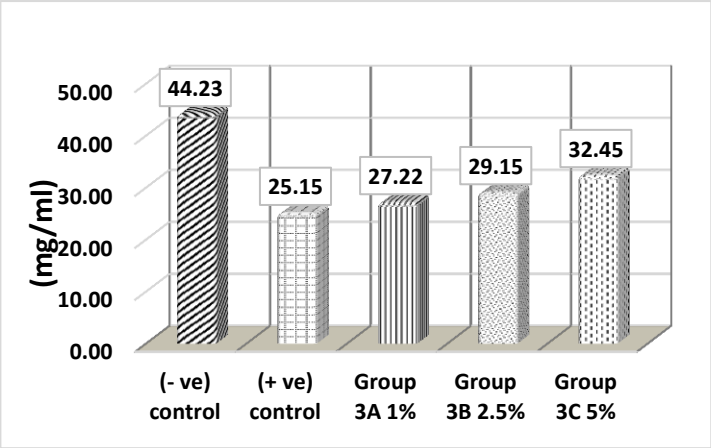

Figure 8: Effect on glutathione peroxidase of rat groups fed Humulus lupulus pre-B[a]P treatment in comparison to control rat groups.

4. Discussion:

In the last years, there has been growing interest in natural substances that can protect liver damage caused by various contaminants that human are exposed to. This study represented an assessment of *humulus lupulus* nutritional and biochemical effects in rats damaged liver induced by B[a]P.

Benzo[a]pyrene (B[a]P) is a highly toxic polycyclic aromatic hydrocarbon (PAH) present in our environment as a food contaminant and air pollutant, which attacks cellular macromolecules and leads to toxicity and carcinogenicity (Shou *et al.*, 1994). Whereas the liver plays an important role in protecting the organism from potentially toxic chemical insults, including toxic chemicals (Grunhage *et al.*, 2003). The liver is often regarded as a primary target of BaP because this organ contains many of the enzymes involved in bioactivation of the parent molecule. However, 21% of a single BaP dose is available in the liver within 10 minutes, and high B[a]P levels within 30 minutes (Weyand and Bevan, 1986). There have been many attempts to screen phytochemicals with high inhibitory effects on B[a]P toxicity. Among them, about 500 varieties of flavonoids (Havsteen, 1983).

Our results revealed a profound protective effect of hops on liver damage in a dose-dependent manner. Noteworthy, these protective effect occurred despite the B[a]P liver damaged as reflected by serum levels of transaminases, bilirubin and the detoxifying enzymes analysis of the liver in the three main groups that supplemented with different levels of *humulus lupulus* (1.0, 2.5, and 5.0%) compared to the normal control and b[a]P treated rats.

There is already a lot of researches that have concluded the beneficial effects of hops on hepatic injury in vivo and vitro. In our study, the *humulus lupulus* supplementation protocol with pre-B[a]P treatment proved to be the most effective protection against the liver deterioration induced by B[a]P. However, the hops 5.0% supplementation level was more efficient than the others. The results showed significant raised on the examined detoxifying liver enzymes: glutathione, GSH, and GPx, which revealed how potent the hops can be in the stimulation of the detoxifying liver enzymes.

Raised levels of GSH protect cellular proteins against oxidation by detoxification through direct thiol conjugation in addition to detoxify ROS directly, neutralizing reactive intermediate species that caused by xenobiotics exposure, which comprise chemical carcinogens (Ketterer, 1998). Thus, the ability of cells to maintain GSH level is important to protect cellular function and integrity. On the other hand, depletion of GSH enhances renal and hepatotoxcity (Thomas, 1993), and associated with a number of human diseases including liver disease, Alzheimer's disease, cancer, Parkinson's disease, heart

attack, stroke, diabetes as well as infection caused by HIV and AIDS (Wu *et al.*, 2004).

NQO1 and GST are important detoxification enzymes that protect cells from oxidative damage and represent important targets for chemoprevention. Moreover, and in line with our results, a study carried out by Dietz *et al.* (2008) on female rats that orally fed on standardized hops extract (7.5 g extract/kg B.W. per day, containing 2% XN). The study approved hops effects on inducing detoxification enzymes in vivo, that's effect was revealed through significant induction of GST activity in the liver and mammary gland addition to the induction of NQO1 activity. From other side, the antioxidant response element (ARE) and its transcription factor Nrf2 which is repressed under basal conditions by Keap1, regulate the transcription of many detoxification enzymes. On this respect, it has been revealed that XN alkylates Keap1, thus enhance the concentration of free Nrf2 in the nucleus that lead to ARE activation (Dietz *et al.*, 2008).

Interestingly, Ferk et al. (2010) administered XH (71 µg/ kg BW) in the drinking water of rats for 7 days and analyzed the effect on NQO1 and GST activities in the liver. Whereas, there were no significant differences in enzyme activities were detected between the control and XH group. In contrast and regarding the GST activity, XH motivate GST activity in the liver and mammary tissue, therefore it is supposed that other compounds in hops might be responsible for the observed GST induction. The GST enzyme family consists of a variety of GST isozymes that are induced through a variety of different receptors or transcription factors, such as Nrf2, and the aryl hydrocarbon receptors (Higgins and Hayes, 2011).

Once the liver's function of metabolizing nutrients, detoxifying xenobiotics and making blood-clotting proteins in addition to other functions related to metabolism. Hence, when any damage occurs to liver tissues, it leads to leakage of liver enzymes into the blood. AST and ALT are two major enzymes assayed in such conditions. Therefore, their increased level in blood indicates liver damage (Okwu, 2014).

In our present study, there was a significant increase in the AST and ALT concentration in the B[a]P treated rats. This suggests high serum enzyme activity and cellular leakage possibly due to oxidative damage of liver tissues (Mukherjee, 2003). The observed increase in AST activity correlates other works that reported increase in AST activity due to damage to liver tissue (Osman et al., 2009). However, our results showed that supplementation significantly and dose-dependently reduced AST and ALT concentration, particularly in rats supplemented with hops before B[a]P treatment.

The prenyl chalcones in hops are known to have high antioxidant activity when evaluated towards lipid peroxidation in various vitro systems. For example, it was reported that XN protects cultured rat hepatocytes from tert-butyl hydroperoxide (TBH) toxicity (Rodriguez *et al.*, 2001). Moreover, it was reported that hops XN possess a hepatoprotective effect against carbon tetrachloride which induced acute liver injury (Pinto *et al.*, 2012). It is well established that the cleavage of the carbon-chloride bond of CCl4 leads to the formation of a trichloromethyl peroxy radical which is involved in the pathogenesis of liver injury (Cheeseman et al., 1985). Pinto *et al.* (2012) concluded the XN hepatoprotective effect through the significant reduction in relative liver weight and the normalisation of both histopathological alterations and plasma enzymatic lactate dehydrogenase activity (LDH), AST and ALT activities compared to CCl4-treated rats in the absence of XN.

In further study with different protocol that has used model of acute ethanol rats and XN pre-treatment with 0.4mg/kg b.w. concentration. The XN pre-treatment protected AST, ALT and LDH activities by 97.8%, 93.2% and 87.9%, respectively, compared to control rats. On the contrary, the serum AST, ALT and LDH of ethanol treated rats were clearly elevated as 212%, 180%, and 148% respectively compared to control rats. Also there wasn't any significant effect of XN on normal rats. On the other hand, the rats pre-treated with XN in a dose-dependent manner have been protected against reduced glutathione (GSH), glutathione S-transferase (GST) and catalase (CAT) reduction induced by acute ethanol administration. The level of GSH, GST, and CAT concentrations in rats livers reaches 91.2%, 67.8% 80.8% respectively compared to control levels. In agree with our results, Pinto *et al.* (2014) demonstrated that the hops XN administration to rats protects against ethanol acute toxicity.

otherwise, it was found that hops xanthohumol has inhibition effects on the crucial pathogenic steps that lead to chronic liver disease in model of CCl4 toxicity including inflammation and pro-fibrogenic genotype stimulation of hepatic stellate cells. Interestingly, XN also act as hepatoprotective agent in *vivo* model of HCV infected at a dosa of 100mg/kg BW that led to reduce liver fibroses and inflammation in comparison with HCV animal model that hasn't been treated with XN (Weiskirchen *et al.*, 2015). Additionally in this model, XN prevent the occurrence of hepatic steatosis that is associated with preventing the activity of microsomal triglyceride transfer protein (Yang *et al.*, 2013).

Malondialdehyde (MDA) is one of the final products of lipid peroxidation, which reacts with thiobarbituric acid to form a pink chromogen-thiobarbituric acid reactive substance (TBARS). Where the process of lipid peroxidation is generated in organism cells due to the presence of free radicals. Therefore, elevation of free radicals lead to overproduction of MDA which

known as an indicator of oxidative stress and the antioxidant status in cancerous patients (Gawel et al., 2004).

From our results, there was a significant and dose-depended reduction in MDA concentration in hops supplementation groups especially in the hops supplemented group before the rats treated with B[a]P which implies how antagonistic the hops could be to lipid peroxidation. Whereas, the only B[a]P treated groups showed significant elevation in MDA rate. That compatible with Gao et al. (2011) study that demonstrated significant and dose dependent increase in the level of MDA in rats treated with BaP compared to the normal rats. If there is a positive relation between the status of lipid peroxidation (LPO) and genotoxicity (Mayer *et al*., 2000), decreasing MDA rate by hops supplementation indicates that the reduction in lipid peroxidation suggests there protection activity against genotoxicity.

In this study, the serum total bilirubin level was significantly higher in the B[a]P treated rats in comparison to the normal rats, which considered as an indicator of non-alcoholic liver disease as a result of preventing the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes. Or due to excessive heme destruction and blockage of biliary tract. Thus, bilirubin is one of the most accurate analyzes used in the diagnosis of hepatic diseases (Reddy *et al*., 2012). Whereas, the hops administration groups showed significantly and dose-dependentlydecease in serum bilirubin level, which demonstrated the hops efficiency in liver protection by decreasing the level of bilirubin.

By discussing this results, we could find out that hops supplementation have hepatoprotective activity against B[a]P toxicity and that action is through induction of liver detoxification role. Similar to our results, Gerhauser *et al.* (2002) revealed that Xanthohumol characterized as a monofunctional inducer, which selectively induces quinone reductase (QR) a Phase 2 enzyme without simultaneously causing for transcriptional activation of the Cyp1A1 a Phase 1 enzyme. While a latter finding of Harrigan et al. (2005) demonstrated that also Xanthohumol at nanomolar concentrations completely protact HepG2 cells against DNA damage induced by B[a]P which activated by 2-electron oxidation, catalyzed by cytochromes P450 CYP1A1, CYP1A2, CYP1B1 and epoxide hydrolase (EH), to the ultimate mutagen BPDE (BaP-7,-8-dihydrodiol-9,10-epoxide) by a two-step epoxidation. This findings also supported by the study of Dietz et al. (2005) who clarified that induction of QR by xanthohumol is mediated by the activation of transcription factors interacting with antioxidant-responsive element (ARE), which is known to up-regulate numerous detoxifying enzymes, including QR (Talalay, 2000).

Based on previous, xanthohumol possess a beneficial action on detoxifying carcinogens by preventing Phase 1 enzymes and stimulate Phase 2 enzymes, which also represent a target of cancer chemoprevention.

5. CONCLUSION:

This study demonstrated that *Humulus Lupulus* dietary supplements is a potent and efficacious hepatoprotective agent, and have the potential as preventive agents against B[a]P iduced liver damage through induction of detoxifying enzymes. In addition to the nutritionally and immunomodulatory effect. And cleared that *Humulus Lupulus* which grow in Egypt has nutritional and wide medicinal effects.

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