

**Effect of *Humulus lupulus* on Serum Lipids Profile in Adult Albino Rats
Injected With B[a]P**

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تأثير حشيشة الدينار على صورة دهون الدم في فئران الألبينو البالغة التي حققت
بالبنزو[أ] بيرين

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تهدف هذه الدراسة لتقدير تأثير حشيشة الدينار باستخدام نسب مختلفة منها (١.٠، ٢.٥، و ٥.٠%) وذلك على صورة دهون الدم في الفئران. وقد تم استخدام ٣٠ من ذكور فئران الألبينو البالغة في هذه الدراسة بحيث تراوح وزن كل فأر (١٥٠ + ١٠ جم)، وقد تم تغذية الفئران على الغذاء الأساسي لمدة أسبوع واحد قبل بدء التجربة للتكيف. ومن ثم تم تقسيم الفئران إلى خمسة مجاميع رئيسية، المجموعة الأولى تم تغذيتها على الغذاء الأساسي كمجموعة ضابطة سالبة، أما المجموعات الثانية كانت مجموعة ضابطة موجبة تغذت على الغذاء الأساسي مع حقنها بالبنزو[أ]بيرين، أما المجموعة الثالثة تم تغذيتها على الغذاء المدعم بحشيشة الدينار قبل معالجتها بالبنزو[أ]بيرين وقسمت إلى ثلاثة مجاميع فرعية وفقاً لنسبة تدعيم الغذاء بحشيشة الدينار (١.٠، ٢.٥، و ٥.٠%). في نهاية مدة التجربة (٣٥ يوم) تم ذبح الفئران وتجميع الدم وفصل السيرم لإجراء التحليلات البيوكيميائية، وقد خلصت النتائج إلى أن تغذية الفئران على حشيشة الدينار بالنسب الثلاثة المستخدمة (١.٠، ٢.٥، و ٥.٠%) لم يكن له تأثير واضح ومعنوي في صورة دهون الدم وتبين ذلك من خلال معدل الكوليسترول الكلي، الليبوبروتين منخفض الكثافة، الليبوبروتين عالي الكثافة، والجليسريدات الثلاثية. الاستنتاج: أكدت الدراسة أن حشيشة الدينار ليس لديها أي تأثير قوي على صورة دهون الدم في فئران الألبينو البالغة.

الكلمات المفتاحية:

حشيشة الدينار، صورة دهون الدم، البنزو[أ]بيرين

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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 serum lipids profile in adult albino rats injected with B[a]P. 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 for acclimatization before starting the experiment. After one week, rats were divided into three main groups, according to the following scheme for Successive 42 days (each group with 6 rats): Group (1): control negative was fed on basal diet. Group (2): control positive fed on 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 no significant action in reducing Total Cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), nor Triglycerides (TG). In Conclusion: This study demonstrated that *Humulus lupulus* dietary supplements have no effects on serum lipid profile.

Keywords: *Humulus lupulus* – Total Cholesterol – LDL – HDL – TG.

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1. Introduction

Recently, researches have been interested in the nutrients of natural foods for their functional role in the medical field. So far, many vitro and vivo studies have been conducted to prove the importance of natural foods nutrients in the lipid profile. Over all, the studies demonstrated that particular food have the ability to reduce TC, LDL, and TG, also ameliorate HDL levels.

In the 12th century, *Humulus lupulus* is known all over the world as the raw material in the beer industry that gives it flavor and aroma characteristic (Olas et al., 2011). In addition to its ability to preserve beer and prevent spoilage due to their polyphenolic compounds and acyl phloroglucides (Natarajan et al., 2008).

Hops were used in the traditional medicinal as a medical plant since ancient times, that includes treatment of anxiety and insomnia, mild sedative, pain reliever, and for stimulation of the gastric function as combating dyspepsia (Zanoli and Zavatti, 2008).

Hops attracted attention because of their functionality in obesity management that have proven by further study. Obesity develops from an energy imbalance, when energy intake exceeds energy expenditure in the form of food (Tseng et al., 2010) and it is primarily characterized by excessive adiposity (Park et al., 2008). Obesity related to increased risk for several chronic diseases such type II diabetes and cardiovascular disease (Legette et al., 2013).

Legette et al. (2013) determined the effect of oral administration of hops XN on biomarkers of a metabolic syndrome connected with obesity, including atherogenic, dyslipidemia, insulin resistance, impaired glucose tolerance, hypertension, the pro-inflammatory and prothrombotic states in obese male rats. The oral administration of hops XN, at a concentration of 16.9 $\mu\text{M/kg}$ b.w. (body weight) had significantly decrease the body weight, as well XN had a positive effect on glucose metabolism.

Additionally, Miyata et al. (2015) demonstrated that XN is a novel SREBP (Sterol regulatory element-binding proteins) activator that reduces the de novo synthesis of fatty acid and cholesterol. SREBPs are key transcription factors that catalyze the expression of genes involved in fatty acid and cholesterol biosynthesis. Furthermore, in obese mice which were induced by feeding high-fat diet, dietary XN independently suppressed SREBP-1 target gene expression in the liver by repressing its maturation and thus inhibited the development of obesity and hepatic steatosis. Dietary XN reduced lipid absorption from the intestine as well.

Because of the SREBPs function as master organizer of cholesterol and fatty acid biosynthesis, also any aberrant SREBP activity may be linked to metabolic disease (Brown and Goldstein, 1999). Therefore, the suppression of SREBP activation is a

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promising therapeutic approach for metabolic disorders treatment (Walker and Näär, 2012). Another study has proven that hops XN has also the ability to inhibit triglyceride synthesis and apolipoprotein B secretion (Casaschi et al., 2004).

Miranda et al. (2016) highlighted the hops XN role in reducing plasma leptin level in mice model fed high-fat diet. Where leptin is a key regulator of body weight and food intake or energy balance by suppressing appetite. This hormone produced mainly in white adipose tissue and considered an important factor linking obesity and metabolic syndrome (Munzberg et al., 2004), thus the increased circulating leptin is a marker of leptin resistance and a common feature in obesity (Park and Ahima, 2015).

The study by Takahashi and Osada (2017) proved that dietary purified XN exerts anti-obesity effects through the inhibition of pancreatic lipase and α -amylase activities and so suppressing lipid and carbohydrate absorption from the small intestine. Dietary purified XN also promoted fecal excretion of fatty acids and carbohydrate in KK-Ay mice model of obesity-diabetes animal.

In a recent study, hops XN has been concluded to have potential therapeutic implications for obesity by inducing beiging of white adipocytes. Also, following 24 h of XN treatment increased oxygen consumption rate. Further, the role of XN was demonstrated to stimulate lipolysis of mature 3T3-L1 (adipose tissue cell line) and primary human subcutaneous adipocytes, inhibit adipogenesis of maturing adipocytes and suppresses lipid accumulation (Samuels et al., 2018).

It is noteworthy that hops XN has multi-faceted anti-obesity mechanisms and so the potential of becoming a nutritional or pharmaceutical agent to ameliorate several metabolic disorders.

Hyperlipidemia refers to elevated cholesterol concentration, which may or may not be associated with elevated TG concentration (Nelson, 2013). Elevated levels of TGs in plasma or serum is one of the metabolic disorders (Justo et al., 2016). In this regard, it has been showed that hops XN significantly decreased the plasma TG levels in obese mice model that fed high-fat diet (Miranda et al., 2016). The reduction of liver TGs by XN further supports the anti-obesity effects of this compound (Kirkwood et al., 2013).

In other side, elevated levels of circulating LDL are well-known as a major risk factor of atherosclerotic cardiovascular disease, which is the largest causes of premature death and morbidity worldwide (Ellulu et al., 2016). Population studies have concluded that flavonoid consumption in human diet is inversely correlated with mortality from cardiovascular diseases (Hertog et al., 1993). In vitro and animal studies proved that flavonoids have beneficial impacts on parameters associated with atherosclerosis (Mladenka et al., 2010).

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The cardioprotective effect of flavonoids can be attributed to their antioxidant, antithrombotic, anti-inflammatory, hypolipidemic properties, inhibition of lipoprotein oxidation and blood platelet aggregation. In that, higher flavonoid intakes are thought to have the ability to reduce risk of developing cardiovascular diseases (Velayutham et al., 2008).

Miyata et al. (2015) demonstrated that hops XN is highly effective in reducing LDL and total cholesterol in mice plasma that fed diet-induced obesity at dietary level of 0.2% or 0.4% XN, and much lower concentration (0.066% XN) at the study of Miranda et al. (2016). Furthermore, dietary XN decreased the plasma levels of LDL by (80%), interleukin-6 (IL-6) by (78%), leptin by (41%), and reducing inflammatory cytokines, which may contribute to the mitigation of obesity and insulin resistance in these mice without risk of liver injury (Miranda et al., 2018). However, obesity is associated with systemic inflammation in which inflammatory cells are increased systemically also in adipose tissue (Gregor and Hotamisligil, 2011). In addition, the increase production of inflammatory cytokines not only in adipose tissue but also in liver, brain, pancreas and muscle tissue (Kanda et al., 2006).

The number of deaths from cardiovascular diseases is on the rise, as aging is associated with an increased incidence of thromboembolism. Thrombotic disorders have become the major contributor to the global disease burden (Xin et al., 2017).

Furthermore, thrombosis is the leading cause of morbidity and mortality in patients with diabetes mellitus, with a reported 65% of diabetic patients eventually dying from thrombotic diseases (Creager et al., 2003). Cancer-associated thrombosis is also an important cause of morbidity and mortality in cancer patients, even people who reach old age (Geddings and Mackman, 2013).

Platelets are the smallest blood component, that capable to act as a fundamental role in thrombosis and maintaining normal hemostasis (Periayah et al., 2017), which make it a potential target for the development of antithrombotic therapy (Roth et al., 2015). Antiplatelet therapy has been successful in reducing the mortality and morbidity of cardiovascular disease. However, current antiplatelet agents approved by the US FDA have serious side effects, including bleeding episodes, gastrointestinal toxicity, neutropenia, thrombocytopenia and incidence of resistance (Xin et al., 2017).

In this regard, it has been reported that consuming moderate dosage of beer have the ability to reduce the risk of blood clots (Pomp et al., 2008) and protect against thrombosis (Gaborit et al., 2013).

Interestingly, Xin et al. (2017) proved the antithrombotic effects of hops by inhibiting platelet activation without increased bleeding risk which is recognized as a

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major limitation of current antithrombotic therapies. Moreover, the study demonstrated that XN induces Sirt1 expression and thereby decreases reactive oxygen species (ROS) overload, prevents mitochondrial dysfunction, and associated membrane damage at low concentrations. Which considered novel insights into mechanisms of thrombotic diseases with possible therapeutic implications. Herein, we aimed in this study to investigate the effect of *Humulus lupulus* on serum lipids profile in adult albino rats injected with B[a]P.

2. Materials and Methods:

2.1 Materials:

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

2.2 Biological Experiments:

2.2.1 Animals:

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

2.2.2. 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).

2.2.3. 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).

2.2.4 Experimental design:

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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 °C, relative humidity ($55 \pm 5\%$), 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 given in non-scattering feeding cups to avoid loss or contamination of food, water was provided to the rats 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 thirty rats were divided into three main groups, 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 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. Physical parameters:

2.3.1. Assessment of food intake, body weight gain and feed efficiency ratio in rats:

During the experiment period (35 days) food intake (FI) was calculated every other day. The biological values of the different diets were determined through its effect on body weight gain (BWG) and feed efficiency ratio (FER) at the end of the experimental period using the method described by (Chapman et al., 1959) by the following formulas:

$$\text{BWG} = \text{Final body weight (g)} - \text{Initial body weight (g)}$$

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$$\text{BWG \%} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{FER} = \frac{\text{body weight gain (g)}}{\text{Food consumed (g)}}$$

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° C till analysis of parameters later.

All serum samples were analyzed for determination the following parameters: Total Cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-L), and Triglycerides (TG).

2.3.3. Estimation of lipid profile:

Total cholesterol (TC) was estimated in the serum according to Allain *et al.*, (1974). Triglycerides were estimated in serum using the method of Carr *et al.* (1993). Determination of serum HDL-cholesterol was by Gordon and Amer, (1977) methods. LDL-cholesterol was determined by using the following equations according to Friedewald *et al.* (1972):

$$\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{TG}/5)$$

At the end, obtained results were expressed as Mean \pm 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:

3.1. Effects on body weight gain, food intake and FER 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 (1) and figures (1, 2, and 3) show the effect of three doses (1.0, 2.5, and 5.0%) of *Humulus lupulus* supplementation on body weight gain, daily

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food intake and food efficiency ratio in pre-benzo[a]pyrene treatment groups when compared with normal and positive control ones that was treated with benzo[a]pyrene.

By comparing the data shown in table (1) and figures (1, 2, and 3) it could be observed that the range of body weight gain in normal rat groups was 1.88 ± 0.11 gm while in case of all B[a]P treated rat groups; the mean of body weight was very low than that of normal group. Control B[a]P groups had mean range of body weight as 0.55 ± 0.10 gm. Whereas *Humulus lupulus* Supplementation groups with (1.0, 2.5 and 5.0%) that pre-treated with B[a]P reflected increasing in the body weight gain to be 0.79 ± 0.14 , 1.18 ± 0.17 and 1.25 ± 0.35 gm respectively. This finding indicated that there was a significant action of the used levels of *Humulus lupulus* supplementation in body weight in examined pre-B[a]P treated rats.

Food intake was significantly higher in normal rats that was 14.2 ± 1.1 g. While there was a significant reduction in food intake in the control B[a]P treated rats and the value was 9.8 ± 1.3 gm. Food intake values of *Humulus lupulus* supplementation groups with 1.0, 2.5 and 5.0% that pre-treated with B[a]P were 10.2 ± 2.3 , 11.0 ± 1.6 and 13.5 ± 2.5 gm respectively. Increased food intake is associated with *Humulus lupulus* supplementation level that could be associated with the *Humulus lupulus* palatability.

Highest food efficiency ratio (FER) 0.75 ± 0.03 , was observed in normal control rat groups. There was no significant difference in FER in supplemented rat groups. This could indicate that used supplementation level (1.0, 2.5 and 1.0%) of *Humulus lupulus* has the same effect toward food efficiency ratio. Whereas, the FER in B[a]P control group was the lowest level 0.44 ± 0.02 that would explain the reduced body weight gain in this group.

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Table 1: Effects on body weight gain, food intake and FER 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	Group 3B	Group 3C
			1%	2.5%	5%
Body Weight Gain (gm/day)	1.88 ± 0.11 ^a	0.55 ± 0.10 ^c	0.79 ± 0.14 ^c	1.18 ± 0.17 ^b	1.25 ± 0.35 ^b
Food Intake (gm/day)	14.2 ± 1.1 ^a	9.8 ± 1.3 ^c	.2 ± 2.3 ^c ·1	.0 ± 1.6 ^b ١1	.5 ± 2.5 ^b ٣1
Food Efficiency Ratio	0.75 ± 0.03 ^a	0.44 ± 0.02 ^c	0.50 ± 0.05 ^c	0.55 ± 0.02 ^b	0.62 ± 0.04 ^c

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

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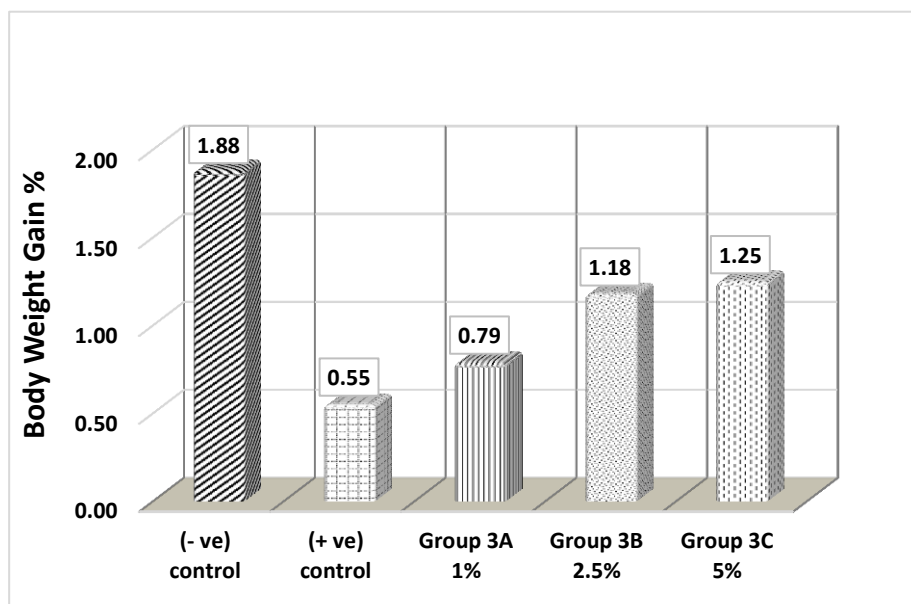


Figure 1: Effect on body weight gain of rat groups fed *Humulus lupulus* pre-B[a]P treatment in comparison to control rat

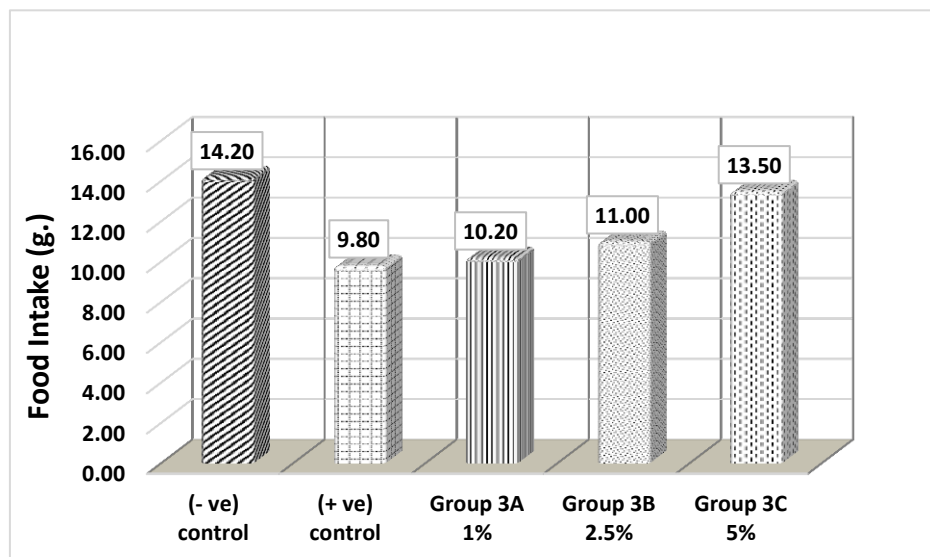


Figure 2: Effect on food intake of rat groups fed *Humulus lupulus* pre-B[a]P treatment in comparison to control rat

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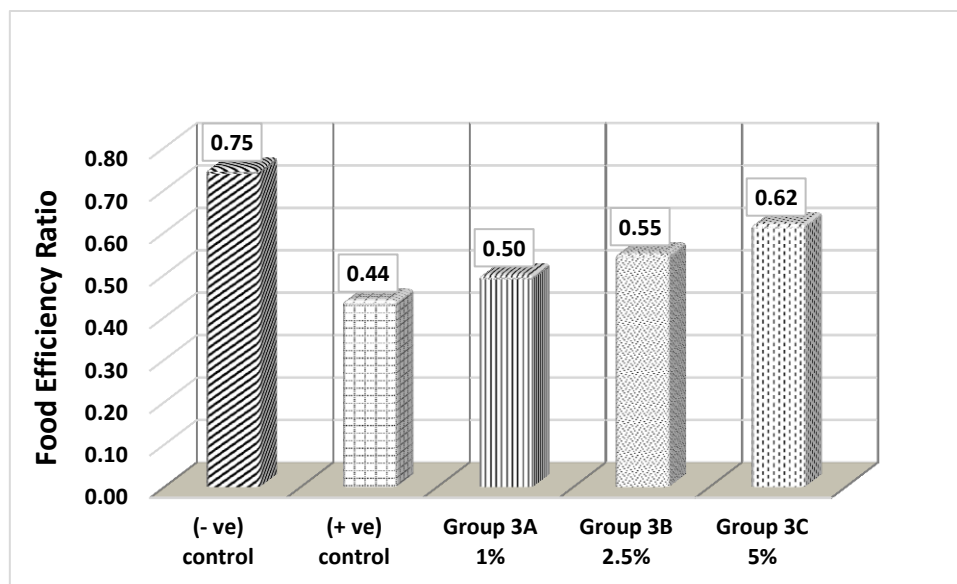


Figure 3: Effect on food efficiency ratio of rat groups fed *Humulus lupulus* pre-B[a]P treatment in comparison to control rat

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3.2. Effects on lipid profile of rat groups fed *Humulus lupulus* before benzo[a]pyrene treatment (pre-benzo[a]pyrene treatment) in comparison to control rat groups:

Data of table (2) and figures (4, 5, 6, and 7) indicated that there is no significant action on lipid profile (total Cholesterol, triglyceride, LDL-C and HDL-C) in positive control rats that treated with B[a]P. As well, from these data it could be observed that there were no significant differences in lipid profile in pre-B[a]P treated groups at all *Humulus lupulus* supplementation levels (1.0, 2.5 and 5.0).

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

Table 7- Lipid profile of rat groups fed *Humulus lupulus* in comparison to control rat groups

Parameters	Group 1 (-Ve Control Group)	Group ٢ (+Ve Control Group)	Group ٣		
			Group 3A	Group 3B	Group 3C
			1%	2.5%	5%
Total cholesterol (mmol/l)	4.57 ± 0.55 ^a	4.54 ± 0.12 ^a	4.58 ± 0.25 ^a	4.57 ± 0.29 ^a	4.56 ± 0.11 ^a
Triglyceride (mmol/l)	1.35 ± 0.10 ^a	1.37 ± 0.12 ^a	1.32 ± 0.11 ^a	1.38 ± 0.10 ^a	1.35 ± 0.11 ^a
LDL-C (mmol/l)	2.70 ± 0.15 ^a	2.74 ± 0.15 ^a	2.70 ± 0.10 ^a	2.71 ± 0.25 ^a	2.74 ± 0.10 ^a
HDL-C (mmol/l)	1.56 ± 0.17 ^a	1.52 ± 0.15 ^a	1.53 ± 0.11 ^a	1.55 ± 0.13 ^a	1.55 ± 0.10 ^a

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

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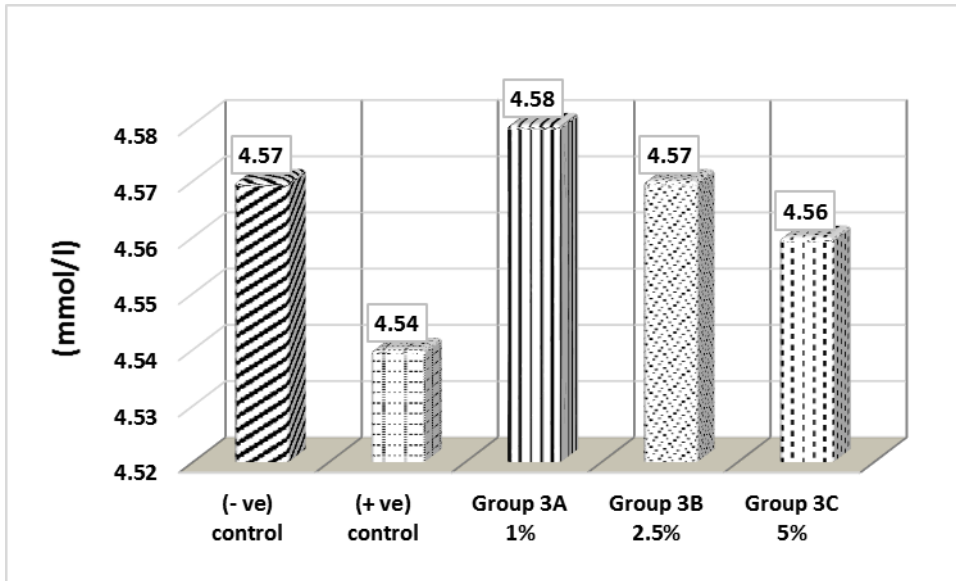


Figure 4: Effect on total cholesterol of rat groups fed *Humulus lupulus* pre-B[a]P treatment in comparison to control rat

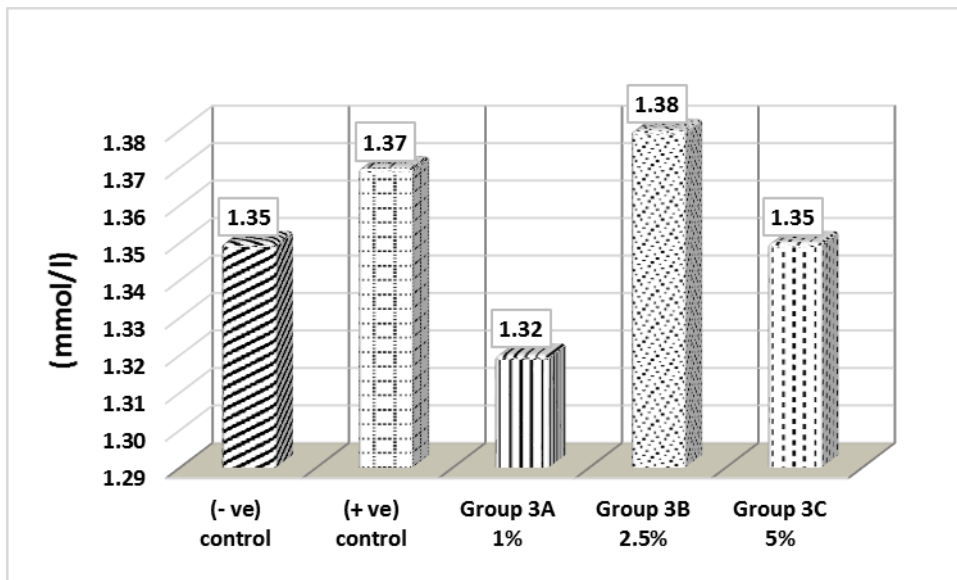


Figure 5: Effect on triglyceride of rat groups fed *Humulus lupulus* pre-B[a]P treatment in comparison to control rat

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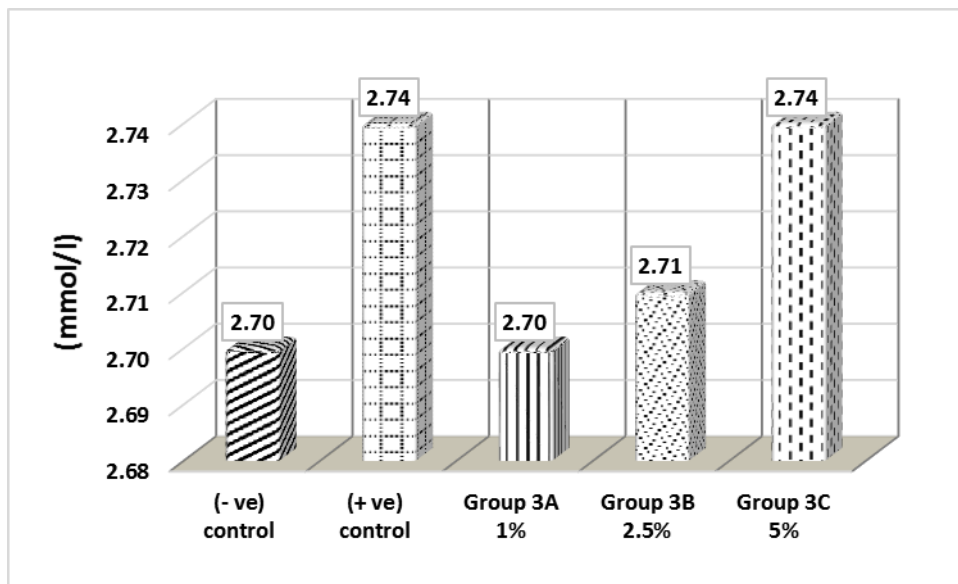


Figure 6: Effect on LDL-C of rat groups fed *Humulus lupulus* pre-B[a]P treatment in comparison to control rat groups.

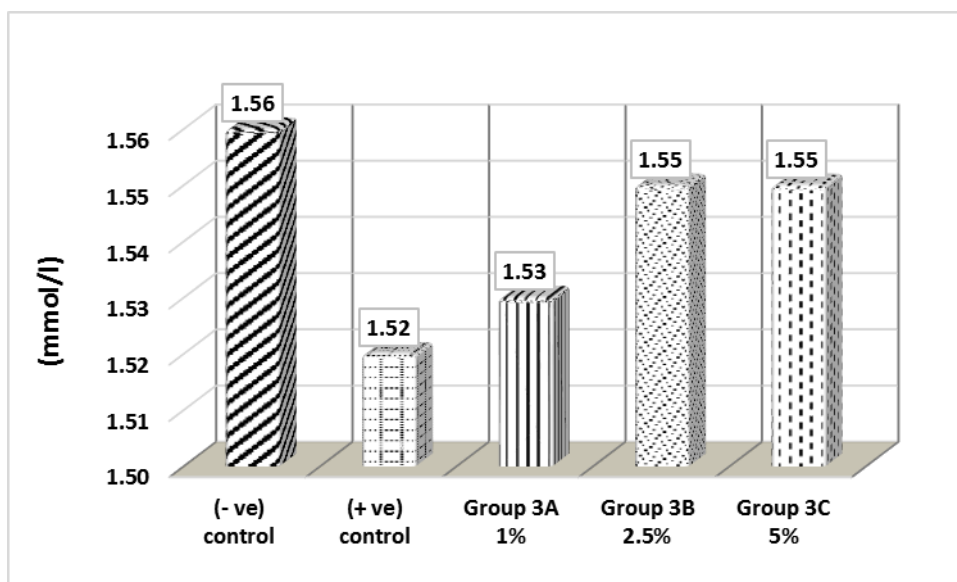


Figure 7: Effect on HDL-C of rat groups fed *Humulus lupulus* pre-B[a]P treatment in comparison to control rat groups.

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4. Discussion:

Since hops show various biological activities, we have tested the hops supplementation effect on lipid profile, whereas the results didn't show any significant effect on lipid profile including total cholesterol, LDL, HDL, and triglyceride.

In this regard, these data reveal apparent paradox, whereby our results of hops supplementation haven't showed any improvement effects on lipid profile, despite further studies have indicated that effect. Yajima et al. (2005) found that hop extract and isohumulone inhibit obesity that induced by high-fat diet; this effect is due to the inhibition of intestinal absorption of dietary fat through preventing pancreatic lipase in rodents. Also, controlling of liver cholesterol, blood levels of lipid and TAG concentrations might involve the activation of PPAR α (Miura et al., 2005), However, PPAR α is an important regulator of lipid metabolism and stimulation of PPAR α are used to treat hyperlipidaemia (Kersten et al. 2000).

In addition, Sumiyoshi and Kimura (2013) reported that extracts of hops has inhibited obesity in mice by feeding a high fat diet for a long period. They proposed that specific hop extracts have potential effects on inhibiting obesity and glucose intolerance caused by a high fat diet. Noteworthy, Miranda et al., (2016) has demonstrated that XN administration at 60 mg/kg/day led to lower serum levels of LDL and leptin hormone by 80%, and 41%, respectively comparing to the control rats. Thus, dietary XN present a reduction in body weight gain induced by a high-fat diet.

In further study of Samuels et al., (2018), XN showed anti-obesity effects through catalyzing being as well as reducing adipogenesis and motivating lipolysis. The anti-obesity effects of XN partially intermediated by AMPK signaling pathway which indicates the potential therapeutic implications of XN for obesity.

Beyond these observations, we could indicated that present hypothesis theory explanations of preventing hyperlipidemia and obesity effects of hops is attributed to its mechanism of action on inhibiting of intestinal dietary fat absorption and activation of PPAR α in addition to reduction of leptin and LDL levels.

5. CONCLUSION:

This study demonstrated that *Humulus lupulus* dried cones dietary supplements have no significant effect on serum lipid profile in adult albino rats which injected with B[a]P.

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