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Astaxanthin Extract Potentiates the Functional Performance of Hepatic and Renal Tissues and Can Prevent or Alleviate Hepatotoxicity and Nephrotoxicity Induced by Acetaminophen

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ABSTRACT

The study performed in the animal house of College of Education for girls from the period of 14/9/2020 to 13/1/2021 to assess the anti-oxidant efficiency of Astax. (Astaxanthin) extract against the hepatotoxicity and nephrotoxicity of acetaminophen, twenty of albino male rats were used in this trial, with age of 12 weeks and their weights ranging between 215 to 230 g. The laboratory animals, randomly divided into four categories, five rats for each group. The dosing was orally and once a day, for twenty-one consequent days, the first group was control treated with normal saline 0.9%,and the second was given acetaminophen drug 1000 mg/kg, the third submitted to acetaminophen 1000 mg/kg +Astax. extract 750 mg/kg, while the last (fourth) group received Astax. extract 750 mg/kg only. The study including, the estimation of some biochemical parameters as Aspartate aminotransferase AST, Alanine aminotransferase ALT, and Alkaline phosphatase ALP activity, erythropoietin (EPO) level, and kidney functions (creatinine and urea levels). The findings of statistical analysis have been revealed a significant elevation ($P< 0.05$) in the effectiveness of the hepatic enzymes (AST,ALT and ALP), creatinine, and urea, in the contrast, there was a notable decrease ($P< 0.05$) in the erythropoietin levels in the drug group (acetaminophen 1000 mg/kg) in compared to the control and two other groups. Whereas, the data of the current study exhibited an effective role of Astax. extract to ameliorate the functions of kidneys, hence there was a significant decrease ($P< 0.05$) in urea level conversely, an observable elevation ($P< 0.05$) in the erythropoietin hormone in the group that submitted to Astax. extract when compared with the control group. **The conclusion**, the Astax. extract had a strong capacity to inhibit or reduce the toxicity that caused by acetaminophen drug due to its vital properties and active chemical contained in particular the anti-oxidants.

KEYWORDS

Astaxanthin Extract Potentiates The Functional Performance.

Introduction

The acetaminophen drug has been commonly used for many treatments especially pain and fever all over the world [1,2].On the other hand, it's using can cause necrosis of hepatocytes in related with the dose, the toxicity of this drug resulted from its metabolism in the hepatic and extra-hepatic tissues[3].Moreover, in the USA the hepatotoxicity, as well as acute liver failure have been diagnosed in half of all pathological cases[4].Only small amount of acetaminophen is oxidized by the enzymatic system (cytochrome P450) to give highly toxic chemical metabolite that is N-acetyl-p-benzoquinone imine (NAPQI)[5]. In the normal state, the NAPQI compound is detoxified through conjugation process with glutathione molecules to form two conjugates, cysteine and mercapturatic acid, then both are excreted in the urine [6]. Sometimes, no sufficient amount of glutathione particles are available, mainly in

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acetaminophen overdose or in a deficiency of glutathione, NAPQI toxic metabolite reacts with all of the biological molecules of cellular membranes causing severe hepatic necrosis subsequently [5]. In regard with Astax, it is a natural keto-carotenoid pigment belongs to xanthophylls family [7,8], because of its unique chemical structure Astax. exerts numerous biological features particularly powerful anti-oxidant, inflammatory, cancer, diabetic, and apoptotic potentialities[9,10].

The aim of this trial is to evaluate the possible hepatoprotective and nephroprotective efficiency of Astax. extract against the toxicity that induced by acetaminophen overdose.

Material and Methods

1. Preparation of the Experimental Animals

The present study carried out in the animal house of Biology department/"College of Education for girls", twenty males of albino rats return to the strain Sprague – Dawley, their weights ranging from 215 to 230 g were equipped by the (National Center) of Control and Pharmaceutical Research/Baghdad. The laboratory animals was provided with water and feed that enriched with proteins, when the rats completed three months of ages (sexually matured) the experiment was started.

2. Preparation of Acetaminophen Drug

Drug of acetaminophen with the dose of 1000 mg /kg[11] was purchased from a pharmacy in Al- Najaf Al-Ashraf, then the stock solution was prepared and each male given the suitable concentration according to its weight.

3. Preparation of Astax. extract

In relation to Astax. extract, the concentration of 750 mg /kg was solved in 10 ml of distill water to get the stock solution, then according to the total body weight of the animal the proper dose was given by gavage.

4. The Study Groups

- Control group was comprised five male rats orally given normal saline 0.9% for 21 days without interruption, once a day.
- Second group including five males, also orally submitted to acetaminophen 1000 mg/kg for 21 days continuously, one time at day.
- Third group was composed of five males treated with 1000 mg/kg of acetaminophen drug +750 mg/kg of Astax. extract sequentially for twenty one days, once a day.
- Fourth group, contained 5 males was orally received 750 mg/kg of Astax. extract only also for 21 days once daily.

5. Laboratory Animals Sacrificing and Collection of the Blood Samples

When the experiment was finished, the animals had been anesthetized by using diethyl ether and the blood taken through the heart puncture, so 5ml of the blood put in the gel tube for the biochemical estimation.

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6. The Biochemical Evaluation of Blood

A. Assessment of AST Activity in the Serum

AST effectiveness was evaluated according to [12], and the kits that used in this research was purchased from (Syrbio), Syria company, and the absorbance read at 546 nm.

B. Assessment of ALT Activity in the Serum

According to the methodology of [12], the activity of ALT enzyme was estimated in serum and the kits, as well as the chemical reagents brought from (Syrbio), Syria company. In relation with absorbance, was at 546 nm.

C. Assessment of ALP Activity in the Serum

To evaluate the serum ALP activity, the method that described by [13] was depended, in addition, the chemical kits equipped by Biomerieux company –France. Moreover, the absorbance was read at 510 nm.

D. Assessment of Erythropoietin (EPO) Level in the Serum

Regarding to the erythropoietin hormone level, the method of [14] was used, and the kits, as well as all reagents were purchased from (IBL-International GMBH), Germany company. In addition, the Bio-Elisa reader used in this estimation, and the absorbance was 450nm.

E. Assessment of Creatinine Level in the Serum

In relation with serum creatinine level, the method that mentioned by [15] was used, all the kits and reagents brought from (Biolabo), France company. Furthermore, the absorbance read at 490 by using of the spectrophotometer.

F. Assessment of Urea Level in the Serum

The estimation of serum urea level was according to the principle of scientific method of [15],and the chemical kits, as well as the reagents were equipped by (Biomerieux), the France company, besides at 580 nm the absorbance was read.

7. The Findings Statistical Analysis

Concerning the analysis of study results, the Statistical Package Social Sciences (SPSS),version22 and (ANOVA) test were used in the study, and to extract the significant differences between all the treatments the LSD at P-value (<0.05) was depended in this trial [16].

Results

The statistical data of this study pointed to a remarkable elevation ($P<0.05$) in the activity of liver enzymes (AST, ALT, and ALP) in the group was orally given 1000 mg/kg of acetaminophen in compared to the other groups. In addition, no significant changes ($P>0.05$) had been noticed in these biomarkers when the residual groups of study compared with each other's, as shown in table (1).

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Table 1: Effect of treatment with acetaminophen and Astax. on the serum hepatic enzymes activity

Groups of study	Mean ± SD			
	Number of samples	AST unit / liter	ALT unit / liter	ALP unit / liter
G1	5	1.14 a ±38.40	1.67 a±51.40	3.03 a±151.80
G2	5	3.65 b±83.40	3.65 b±115.40	10.26 b±286.20
G3	5	1.48 a±39.20	3.35 a±52.80	3.03 a±153.20
G4	5	1.48 a±37.20	0.84 a±49.50	1.48 a±150.20
LSD		2.922	3.547	7.519
Sig.		0.0001	0.0001	0.101

*Different letters mean significant differences between the experimental groups at P-value <0.05.

In related with erythropoietin hormone (EPO), there was a significant reduction (P<0.05) in its level at the group that administrated with acetaminophen drug when compared with the other experimental groups. Conversely, the study group that orally subjected to Astax. extract recorded a noticeable raise (P<0.05) in this vital index in comparison to the other groups. Moreover, no observable variation (P>0.05) in the erythropoietin level was reported between other groups of experiment, as noted in table (2).

Table 2: Effect of treatment with acetaminophen and Astax.on the serum EPO level

Groups of study	Mean ± SD	
	Number of samples	EPO mmol / ml
G1	5	15.09±0.19 a
G2	5	9.07±0.98 b
G3	5	15.04±0.32 a
G4	5	17.06±1.14 c
LSD		1.038
Sig.		0.0001

*Different letters mean significant differences between the experimental groups at P-value <0.05

Concern with creatinine and urea levels, there was a significant increment (P<0.05) obtained in the acetaminophen group in compared to other trial groups, table (3).

In contrast to what have been preceded, urea level was significantly decreased (P<0.05) in the group that submitted to Astax. extract when compared with other groups, table (3).

Furthermore, no marked alterations (P>0.05) were revealed in these biochemical parameters between the others.

Table 3: Effect of treatment with acetaminophen and Astax. on serum creatinine and urea levels

Groups of study	mean ± SD		
	Number of samples	Creatinine mg / dl	Urea mg / dl
G1	5	0.25±0.05 a	16.20±1.30 a
G2	5	1.07±0.09 b	43.40±2.41 b

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G3	5	0.27±0.05 a	17.80±1.30 a
G4	5	0.22±0.04 a	13.60±1.82 c
LSD		0.081	2.370
Sig.		0.0001	0.0001

*Different letters mean significant differences between the experimental groups at P-value <0.05.

Discussion

According to the finding of this study, there was a marked increase in the effectiveness of liver enzymes including AST, ALT, and ALP in the group of animals that treated with acetaminophen drug when compared with other experimental group, was similar to the result of many studies [6,17]. It likely to be due to the elevated production rate of acetaminophen reactive metabolite NAPQI that resulted from the high dose of drug which may consequently lead to cellular glutathione consumption and targets various proteins molecules particularly the mitochondrial proteins that causes oxidative stress stimulation, subsequent mitochondrial dysfunction, DNA damaging, and finally cellular necrosis, as confirmed by some studies[18], which may be followed by liver enzymes releasing to the blood stream, hence their activity significantly increased.

The increased activity of liver enzymes possibly attribute to the pathological influences of drug to the hepatic tissues through disturbance of the metabolic processes and hypertrophy of the hepatocytes, which may cause stress to the rough endoplasmic reticulum to produce large amount of liver enzymes, and then by changing the selective property of cellular membranes these enzymes can release directly to the blood and their effectiveness will increase accordingly. Moreover, the elevated levels of liver enzymes that transported of amine group in the serum due to acetaminophen drug may be explained to the structural damage of hepatic cellular membranes, which led to enzymes leakage to the circulation, so the increased levels of these enzymes in the blood point to the degree of liver tissue infection, as reported by[6]. Furthermore, the increment in these vital markers might suggest to a reduction in blood perfusion of hepatic tissues due to the drug deteriorative effects, which result in numerous abnormal changes especially in the selective permeability of the cellular membranes and subsequent exit of liver enzymes from the cytosol to the blood.

Besides, the ALT enzyme responsible for the transformation of alanine amino acid to glucose, which transports from the liver to other organs, however the levels of this enzyme are low in the normal conditions, and the increased levels indicate to liver destruction because it particularly available in the hepatic tissues, as demonstrated by some studies[19].

Otherwise, the Astax. extract exerted an effective role to preserve the cellular structure of the hepatic tissues and all the cytoplasmic, as well as nucleic constitutes, in addition to the selectivity of cellular membranes, thereby the liver enzymes did not liberate to the circulatory system in the study group was orally administrated with acetaminophen plus Astax. extract when compared with the drug only group. Overall, Astax. acts through its anti-oxidant, lipid peroxidation, and autophagy activities, as well as the inhibition of hepatic stellate cells stimulation, in addition, reducing the production of damaging reactive oxygen species, and conversely enhancing the synthesis of nuclear erythroid 2-related factor 2 that is the main transcription factor for endogenous defense system of anti-oxidants to support the hepatic tissues [20], thus it's possible the Astax. extract works by all these mechanisms together to protect the liver and maintain the effectiveness of its enzymes.

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As for the group of animals that received Astax. extract only, no alteration in the AST, ALT, and ALP activity had been recorded, the finding agrees with some studies [21]. It probably due to the unique potentialities of extract in particular the anti-oxidant capacity which prevents the production of harmful free radicals and inflammatory cytokines, as well as inhibits liver tissue fibrosis, as proved by some studies[22], so the anti-fibrogenic influences of the Astax. may be induced during this study. On the other hand, the present trial pointed to a notable decrement in the erythropoietin level in the males were orally given acetaminophen. The result may be explained to the negative impact of this drug to the glomerular filtration process through the stimulation of high toxic oxygen species synergistically with the patho-histological variations that caused by acetaminophen in the renal tissues which consequently lead to decreased biological synthesis of erythropoietin, as proposed by some studies[23].

It likely to be due to a fibrosis of peritubular interstitial cells and subsequent decrease of erythropoietin level substantially, as confirmed by [24], when they used 500 mg/kg of erythromycin antibiotic twice a day for 14 sequential days. In addition, the reduced level may suggest to a possible suppression of sensitivity mechanism of oxygen level in the renal cells, and then inhibition of the stimulating hypoxia inducible factor (HIF), which regulates the transcription process of hormone gene in the kidney tissues, that causes a decrement in the erythropoietin-producing rate [25]. In the contrast, there was a noticeable improvement in the erythropoietin level revealed in the group that orally dosed with acetaminophen +Astax. extract in comparable to the group which subjected to the drug only .The finding possibly attribute to the potent properties of the carotenoid compound (Astax.) including strong anti-oxidant and inflammation, as well as anti-cellular death, hence it possesses the ability to scavenge the detrimental free radicals [26], in addition to reduce the degenerative and necrotic effects of this type of drugs.
Lastly, the erythropoietin level was markedly increased in the experimental group that submitted to Astax. extract only in comparison to other study groups .This can explain to the vital activities of the fat-soluble terpeny compound, it has two functional groups in its chemical structure; keto and hydroxyl, in addition to the conjugated double bonds, therefore it more potent than vitamins C and E approximately 65, and 50 times to protect the cellular membranes, besides the Astax. more effective than other carotenoids and food elements to attack the single oxygen radical because it exhibits anti-oxidant activity stronger than co-enzyme Q, green tea, beta-carotene, and lutein about 800,550,11 and 2.75 times respectively as postulated by some studies[27],thus the erythropoietin level notably increased in the blood.
With regard to creatinine and urea, the current study showed a significant raise in these criteria in the laboratory animals that administrated with acetaminophen was compatible with many studies [11,28].It's possible due to the renal tissues destruction because of the drug negative impacts which may lead to severe glomerular damage, alteration of glomerular capillary permeability, nephrotic dysfunctions, and consequent disturbance of glomerular filtration mechanism, thereby accumulation of creatinine and urea in the blood and increase their levels remarkably. Concerning to creatinine clearance, it’s a good index to assess the functional performance of renal tissues in the clinical cases, as was proved by numerous studies [29,30].

The elevated level of creatinine in the males that treated with acetaminophen perhaps attribute to renal tissues erosions, and a reduction of blood perfusion, or may be suggested to an inflammation, or blockage of the urinary tract [31] due to the toxic influences of various free radicals especially hydroxyl and hydrogen peroxidase which stimulated by the drug dosing. In relation to urea, it is increased in some pathological states including decreased glomerular

filtration rate as in acute and chronic renal failure, as well as kidney diseases [32], that may occur during this experiment and led to a raise in the urea level substantially. In the opposite of what have been foregoing, creatinine and urea were significantly decreased in the group of rats that given acetaminophen, then Astax. extract together when compared with the drug group only. It likely attribute to the preventive role of this extract to preserve the kidney tissues under different pathological conditions [33], as high generated necrotic free radical, renal tubules ischemia, direct tubular toxicity, which may cause hypoxia to the medulla tissues [34], thus the effective chemical contents of extract synergistically act to protect the kidney nephrons from the damaging impacts of acetaminophen.

Finally, as for the study group that orally received Astax. extract, there was a remarkable decrement in urea level in comparison to the other residual groups, might explain to the potent scavenging capacity of the extract, hence it inhibits or decreases all the oxidative stress processes which arise from various reactive oxygen species in particular nitric oxide, nitric peroxide, and subsequent lipid peroxidation, therefore the Astax. has an important physiological role to protect the renal tissues from many dangerous diseases [35], because of its strong anti-oxidant, degenerative, fibrotic, hemorrhagic and cellular necrotic properties.

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