Physiological study of the effect Astaxanthin (shrimp extract) on some biochemical markers in male rats induced by Formaldehyde

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Abstract: The present study was conducted to show the protective and treated effects of astaxanthin in male rats induced by formaldehyde. The total numbers of male rats include ninety one (91) males, weighing (190-300) mg, aged (15-17) weeks. The animals were housed in animal house of college of science / University of Kufa from period February 2018 to July 2018. The animal were divided into two groups protective group total male rats were (36) and treated group (55) male rats.

The result sowed a significant increase (p< 0.05) in Asparatate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) levels in formaldehyde group whene compared with control group and significant decrease (p< 0.05) in protective and treated groups of astaxanthin 250 and 500 mg/kg as compared with control group and formaldehyde groups. The study of biomarkers also showed a significant increase (p< 0.05) in Regucalcin (RUG), 8-hydroxy-2 deoxyguanosine (8-OHGD) and Myeloperoxidase (MPO) levels in formaldehyde group as compared with control group and showed a significant decrease (p< 0.05) in all biomarkers when compared of astaxanthin & formaldehyde with formaldehyde group and non-significant difference (p<0.05 when compared with control group).

The present study roles of Astaxanthin as protective and treatment were documented by decrement by liver enzyme AST, ALT and ALP also some biomarkers also Creatinine after increment by formaldehyde.

Keywords: Formaldehyde, Biomarkers, Astaxanthin, Male rats.

1- Introduction

Astaxanthin is a member of the xanthophylls because it contain not only carbon and hydrogen but also oxygen, consists of two terminal rings joined by polyene chain and contain two asymmetric carbon atom located 3,3 position position of $\beta$-ionone ring with hydroxyl group it a carotenoid with powerful antioxidant properties that exists naturally in various plants, algae, and seafood. The purpose of the present study is to examine the protective action of astaxanthin against high-glucose-induce oxidative stress, inflammation, and apoptosis in proximal tubula epithelial cell (PTECs) [1]. Astaxanthin effectively suppressed lipid peroxidation, total RS, $^*$O(2), NO*, ONOO (-), iNOS and COX-2 protein levels, NF-kappaB nuclear translocation, and proapoptotic Bax, whereas it increased anti-apoptotic Bcl2 protein levels [2].

Carotenoids have shown potential antioxidant properties which inhibit free radical formation in various diseases [3]. Formaldehyde is naturally produced in very small amounts in our bodies as a part of our normal, everyday metabolism and causes us no harm, it can also be found in the air that...
we breathe at home and at work, in the food we eat, and in some products that we put on our skin [4]. A major source of formaldehyde that we breathe every day is found in smog in the lower atmosphere and the structural formaldehyde (CH2O) is an important chemical for the global economy, widely used in construction, wood processing, furniture, textiles, carpeting, and in the chemical industry. It has been classified as a human carcinogen that causes nasopharyngeal cancer and probably leukemia [5]. Many cases of poisoning, allergy, asthma, pulmonary damage, cancer and death were reported as a result of formaldehyde exposure from contaminated foods, drinking water, and polluted indoor air [6]. Previous study showed a readily absorbed of F.A. form gastrointestinal tract and hepatotoxicity also histopathological alteration in liver and gastrointestinal tract [7]. F.A. is a mutagenic and carcinogenic and cause a toxicity at low concentration in all organism [8]. The present study was designed to show the protective and treated roles of astaxanthin against formaldehyde in male rats.

2- Materials and methods:

2.1Animals

Ninety one (91) male rats (Rattus norvegicus) were obtained from animal house of college of science / University of Kufa weighing from (200-250)g and aged (12-15) weeks. The study started from February 2018 to June 2018.

2. 2 Preparation of Formaldehyde

The formaldehyde was obtained from (BDH) company and the dose 10 mg/kg of body weight was selected according to [9].The formaldehyde was given intraperitoneally once a day for 14 day.

2. 3Preparation of Shrimp Astaxanthin

The Astaxanthin was obtained by (Asta Read holding Co. Ltd. Japan). The two doses 250-500 mg/kg of body weight of male rats wear selected according to [10]. The astaxanthin was administrated orally for 14 day after two hour of formaldehyde injection in protective groups, while in treated groups astaxanthin was administrated after 14 days of formaldehyde injection orally for 30 and 45 day.

2. 4 Blood samples

The blood was drawn through heart puncture by using disposable syringe (5 mL), then left at room temperature for clotting, and then centrifuged at (3000 rpm) for (15) minutes, serum was isolated and stored at deep freeze (-20) ºC in Al- Sadar medical city in Al-Najaf Al-Ashraf province until using for measurements biomarkers and liver enzymes.

2. 5 Biomarkers

The assessment of (8-OHDG, MPO and REG. ) rats Elisa kits provided by (abbexa-UK) Sandwich immunoassay technique (enzyme-linked immunosorbent assay – automated microtiter plate.

2. 6 Statistical analysis

Results was expressed as a mean ± standard error (SE) and performed using multivariate ANOVA by Graph Pad Prism® software (Graph Pad Software, Inc., La Jolla, CA, USA) and comparison between groups using t-test. Statistical significance was p≤0.05 [11].

3.Results:
Figure (3-1) Effect of two concentrations of Astaxanthin (250 and 500 mg/kg) on AST level in male rats induced by Formaldehyde for 14 days. The figure (4-1) showed significant increase (p<0.05) in AST level in the concentration in the F.A.14D. (65.500±1.822) compared with control group (30.340±1.257), also revealed a significant decrease (p<0.05) in the concentration of AST in protective group (F.A.&Ast.250 mg/kg, F.A.& Ast. 500 mg/kg, Ast.250 mg/kg and Ast. 500mg/kg) (43.400±1.520, 38.780±1.071, 28.040±1.500 and 27.160±1.413) respectively compared with F.A.14D. (65.500±1.822), also showed significant increase (p<0.05) in the concentration in the (F.A.&Ast.250 mg/kg and F.A.& Ast. 500 mg/kg) (43.400±1.520 and 38.780±1.071) compared with control (30.340±1.257), the results also revealed non-significant (p<0.05) in the concentration in the (Ast.250 mg/kg and Ast. 500 mg/kg) (28.040±1.500 and 27.160±1.413) compared with control (30.340±1.257).

Figure (3-2) Effect of two concentrations of Astaxanthin (250 and 500 mg/kg) on ALT level in male rats induced by Formaldehyde for 14 days. The figure (4-2) showed significant increase (p<0.05) in ALP level in the concentration in the F.A.14D. (43.460±1.028) compared with control group (10.860±0.733), also showed significant decrease (p<0.05) in the concentration in the protective group (F.A.&Ast.250 mg/kg, F.A.& Ast. 500 mg/kg, Ast.250 mg/kg and Ast. 500mg/kg) (27.060±1.402, 23.700±1.456, 9.980±0.361 and 9.380±0.341) compared with F.A.14D. (43.460±1.028), results also revealed non-significant (p<0.05) in the concentration in the (Ast.250 mg/kg and Ast. 500 mg/kg) (9.980±0.361 and 9.380±0.341) compared with control (10.860±0.733).
Figure (3-3) Effect of two concentrations of Astaxanthin (250 and 500 mg/kg) on ALP level in male rats induced by Formaldehyde for 14 days. The figure (4-3) showed significant increase (p<0.05) in ALP level in the concentration in the F.A.14D. (53.040±1.317) compared with control group (20.700±0.943), also showed significant decrease (p<0.05) in the concentration in the protective group (F.A.& Ast.250 mg/kg , F.A.& Ast. 500 mg/kg , Ast.250 mg/kg and Ast. 500 mg/kg) (34.080±1.183 , 30.340±0.862, 19.660±0.462 and 19.020±0.361) compared with F.A.14D. (53.040±1.317). The results also indicated non-significant (p<0.05) in the concentration in the (Ast.250 mg/kg and Ast. 500 mg/kg) (19.660±0.462 and 19.020±0.361) compared with control (20.700±0.943).

Figure (3-4) Effect of two concentrations of Astaxanthin (250 and 500 mg/kg) on Creatinine level in male rats induced by Formaldehyde for 14 days. The figure (4-4) showed significant increase (p<0.05) in creatinine level in the concentration in the F.A.14D. (3.120±0.193) compared with control group (0.506±0.046), and significant decrease (p<0.05) in the concentration in the protective group (F.A.& Ast.250 mg/kg , F.A.& Ast. 500 mg/kg , Ast.250 mg/kg and Ast. 500 mg/kg) (1.100±0.145, 0.820±0.037, 0.340±0.040 and 0.280±0.037) compared with F.A.14D. (3.120±0.193), also showed significant increase (p<0.05) in the concentration in the (F.A.& Ast.250 mg/kg and F.A.& Ast. 500 mg/kg) (1.100±0.145 and 0.820±0.037) compared with control (0.506±0.046), also the results indicated non-significant (p<0.05) in the concentration in the (Ast.250 mg/kg and Ast. 500 mg/kg) (0.340±0.040 and 0.280±0.037) compared with control (0.506±0.046).
Figure (3-5) Effect of two concentrations of Astaxanthin (250 and 500 mg/kg) on 8-OHDG level in male rats induced by Formaldehyde for 14 days. The figure (4-6) showed significant increase (p<0.05) in 8-OHDG level in the concentration in the F.A.14D. (14.949±2.119) compared with control group (7.154±0.807), also found significant decrease (p<0.05) in the concentration in the protective group (F.A.&Ast.250 mg/kg, F.A.& Ast. 500 mg/kg, Ast.250 mg/kg and Ast. 500 mg/kg) (9.029±0.646, 7.813±0.698, 8.251±0.785 and 6.713±0.456) compared with F.A.14D. (14.949±2.119), and significant increase (p<0.05) in the concentration in the (F.A.&Ast.250 mg/kg and F.A. & Ast. 500 mg/kg) (9.029±0.646 and 7.813±0.698) compared with control (7.154±0.807), also showed nonsignificant (p<0.05) in the concentration in the (Ast.250 mg/kg and Ast. 500 mg/kg) (8.251±0.785 and 6.713±0.456) compared with control (7.154±0.807).

Figure (3-6) Effect of two concentrations of Astaxanthin (250 and 500 mg/kg) on MPO level in male rats induced by Formaldehyde for 14 days

The figure (4-7) showed significant increase (p<0.05) in MPO level in the concentration in the F.A.14D. (11.114±1.575) compared with control (3.156±1.129), also significant decrease (p<0.05) in the concentration in the protective group (F.A.&Ast.250 mg/kg, F.A.& Ast. 500 mg/kg, Ast.250 mg/kg and Ast. 500 mg/kg) (6.279±1.625, 4.374±1.672, 3.537±1.259 and 3.181±1.988) compared with F.A.14D. (11.114±1.575), also showed significant increase (p<0.05) in the concentration in the (F.A.&Ast.250 mg/kg and F.A. & Ast. 500 mg/kg) (6.279±1.625 and 4.374±1.672) compared with control (3.156±1.129), and non-significant (p<0.05) in the concentration in the (F.A.&Ast.250 mg/kg and F.A. & Ast. 500 mg/kg) (6.279±1.625 and 4.374±1.672) compared with control (3.156±1.129).
mg/kg, F.A.& Ast. 500 mg/kg, Ast. 250 mg/kg and Ast. 500 mg/kg) (6.279±1.625, 4.374±1.672, 3.537±1.259 and 3.181±1.988) compared with control (3.156±1.129).

Figure (3-7) Effect of two concentrations of Astaxanthin (250 and 500 mg/kg) on REG. level in male rats induced by Formaldehyde for 14 days. The figure (4-8) showed significant increase (p<0.05) in REG. level in the concentration in the F.A.14D. (2659±77.863) compared with control group (1451±97.893), also showed significant decrease (p<0.05) in the concentration in the protective group (F.A.& Ast. 250 mg/kg, F.A.& Ast. 500 mg/kg, Ast. 250 mg/kg and Ast. 500 mg/kg) (1756±221.357, 1648±198.708, 1342±110.508 and 1364±80.148) compared with F.A.14D. (2659±77.863), and non-significant different in the concentration in the (F.A.& Ast. 250 mg/kg and F.A.& Ast. 500 mg/kg) (1756±221.357 and 1648±198.708) and (Ast. 250 mg/kg and Ast. 500 mg/kg) (1342±110.508 and 1364±80.148) compared with control group (1451±97.893).

4. Discussion
The present results shows a significant increase in AST, ALT and ALP after induced of male rats by formaldehyde figure (4-1), (4-2) and (4-3). The present study agree with two studies who suggested the elevation of liver enzyme after formaldehyde induced liver injury in rats [12,13]. The study of [14] has been shown that F.A. administration for 2 weeks lead to significant changes in liver tissues due to production of free radicals and reactive oxygen species which can produce damage through the oxidation.

The activation of necrosis by necrosis factor KB (NF-KB) and decrease the level of superoxide dismutase, catalase and glutathione peroxidase have a main role in liver injury and causes elevation in AST, ALT and ALP [15,16] liver enzymes such as AST and ALT released and active after in blood stream after exposure to hepatotoxic compounds and this is liver enzymes may be considered as a markers for hepatotoxicity by many different compounds such as formaldehyde, carbontetrachloride and paracetamole [17,18,19&20].

The elevation in AST, ALT and ALP level may reflect the alteration in the permeability of hepatic membrane also may indicate a severe damage of liver due to changes in liver structural integrity and necrosis. The results of current study figure (4-1), (4-2) and (4-3) has been indicated a significant decrease in AST, ALT and ALP enzymes after astaxanthan treatment.

A little data has been hypothesized the hepatic protective effect of astaxanthin on rats induced by formaldehyde, but there are many studies has been demonstrated the protective effect of astaxanthin on liver cells induced by other toxic compound previous study has been suggested the role of astaxanthin against the hepatic damage by CCL4 and the results estimate the serum enzyme AST, ALT and ALP and showed a significant decrease in the concentrations of these enzymes.
toward the normal level and hue a meliorated effect may be to the roles of astaxanthain as free radical scavenger activity [21&22].

The antioxidant properties which may be very important for hepatoprotective effect of astaxanthin and reduced of ROS because a unique molecular structure of astaxanthin which has a hydroxy one ionene ring and keto on the other rings to scavenge radicals by combining with two none moieties [23].

Other studies has been postulated the effect of astaxanthin on liver injury by reduced the expression of tumor nectrotic factor –α(TNF– α ) and tumor growth factor- β -1) which may be cause inhibited of hepatic injury and decrease AST ,ALT and ALP level [24]. The present data showed a significant elevation in creatinine in male rat induced by formaldehyde and significant elevation in creatinine in male rats induced by formaldehyde and significant decrease in urea concentrations, figure (4-4 ) and (4-5) no previous studies has been suggested the role of F.A on kidney dysfunction, therefore the discussion of these marker (creatinine and urea ) based on kidney. In former study of [25]has shown the elevation of creatinine and decrement in urea concentration after induced by AS2O3 and considered to be a marker of kidney failure .

The low clearance values for creatinine and decrease the ability of kidney to filtrate these waste products also the detection of renal damage and break down of tissues has an important roles in renal injury [26 &27]. The present results showed a significant increase (p< 0.05) in 8-OHdG lever after formaldehyde induced induced in male rats figure (4-6). This study agree with many studies that has been demonstrated that rats exposure to formaldehyde in both inhalation and interpretational injection may lead to enhanced DNA damage by higher level of 8-OHdG that considered a marker of DNA oxidative damage [28&29]. Another studies on laboratory workers exposed to formaldehyde has been observed high level of 8-OHdG in the peripheral blood lymphocyte , also the similar effect of F.A. was showed by increase DNA damage [30,31&32]. Many studies has suggested the mechanism in which 8-OHdG high level in mice and rats induced by formaldehyde and showed that ROS-initialed oxidation stress and induced DNA damage by reaction of the C-8 position of 2-deoxyguanosine to form 8-hydroxy-2-deoxyguanosine (8-OHdG) and therefore cause mispairing during DNA replication or in which convert G to T and G:C become T:A and mutation occur [33&34]. The present study indicate a significant increase (p< 0.05) in (MPO) level in formaldehyde group figure (4-7) .There are no data or research study the effect of formaldehyde on MPO level experimentally or clinically therefore the discussion of this marker will depend on related research on MPO. Several studies has been confirmed the roles of MPO in oxidative stress and the peripheral blood cells such as phagocytic (lymphocytes or neutrophils ) produces large amount of (ROS) by the major pathway include membrane bound NADPH oxidases which produce superoxide with production subsequent hydrogen peroxide and peroxide can be used by phagocytic enzyme (MPO) also the enhance production of superoxide and hydrogen peroxide make myeloperoxidase enzyme more toxic and with high level and hypochorous acid ,superoxide anion can react with nitric oxide (NO) and make high oxidative stress [35&36].

The present data reveal significant increase (p <0.05) in regucalcin level after formaldehyde induced male rats figure (4-8).The researches about the effect of formaldehyde on regucalcin level are an absence , therefore the discussion of this marker depend on the related paper deals with acute liver failure or liver disease on regucalcin . In recent study[37,38,39,40] have demonstrated that regucalcin for the first time or called SMP 30 at high level in the plasma of mice and speculated the abnormal increase for several reasons included an increase in permeability of membrane due to hepatocytes necrosis and damage allowing the regucalcin to release into the blood because SMP 30 is synthesized and expressed in the liver tissue also decrease degradation and clearance from the plasma causes a hepatic injury in addition some factors stimulated other tissue to release regucalcin therefore it be useful for the prognosis of severe hepatic injury .
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