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EFFECT OF ACRYLAMIDE ON THYROID AND LIVER FUNCTIONS IN ADULT MALE RATS

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Abstract

The present study was carried out to investigate the effects of acrylamide (ACR) on thyroid and liver functions and histopathological changes in adult male rats. Twenty adult male rats were used in the present study. The animals were acclimatized for two weeks before beginning of the experiment. The animals were divided randomly into two equal groups. The 1st group (control) administrated distal water orally by gavage, the 2nd was given ACR at a dose of 5 mg/kg BW/day for 45 days. The results revealed a significant increase in serum thyroxin (T_4), triiodothyronine (T_3) concentrations and thyroid stimulating hormone (TSH) was observed in ACR group. Moreover, a significant increase in serum levels of AST, ALT and ALP enzymes in ACR-group. Histopathological study showed the irregular size and shape of thyroid follicles and vacuolation of colloid and liver section appeared congestion of central vein, flattening and vacuolation of hepatocytes in ACR treated group.

Key words: Acrylamide, Thyroid, Liver and Male rats**.**

1. Introduction

Acrylamide is an odorless, white crystalline solid at room temperature and has chemical formula: C_3H_5NO and structure $H_2C{=}CHCONH_2$, and it is a water soluble substance (Asha *et al.*, 2008; Schwend *et al*., 2009). The orally consumed acrylamide is absorbed into the general circulation, then distributed to different organs and reacts with DNA, neurons, essential enzymes and hemoglobin and causes different toxic effect (Rayburn and Friedman, 2010). Acrylamide and polyacrylamide used in many applications such as: plastic, aesthetic surgeries, cosmetic industries, opthalmic operations, laboratory processes and oil recovery processes (Schwend *et al*., 2009). Acrylamide in the small amounts used in sugar beet juice clarification, foundry sand, printing ink emulsion stabilizers, thickening agents (Magdy *et al*., 2013), scientific application of acrylamide as: water purification, gel electrophoresis and sewage treatment for agricultural sprays, textile printing paste and water retention aids, adhesive and binders for seed coating, monomer acrylonitrite used as a raw material in many manufacturing of acrylic fiber, plastics and synthetic rubbers (Woutersen, 1998).

Thyroid gland secretes thyroid hormones (T_4 and T_3). Thes hormones remain fused with thuroglobulin which was stored in the colloid of the thyroid follicles until release into the circulation in response of the thyroid gland to thyroid stimulating hormone (TSH). More than 90

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% of T_4 and T_3 are bound to the protein and small amount of them still free in the circulation and a small amount of them still free in the circulation which are physiologically active form and responsible for regulation of TSH secretion from pituitary through the feedback mechanism (Tietz, 1987). In adult thyroid hormones are playing important role in protein, lipid and carbohydrate metabolism and in heat generation (Kim, 2008), many endogenous or exogenous agents affected thyroid gland function resulted in various subclinical effects (Surks *et al.*,2004) or clinical manifestation (Dallaire *et al.,* 2009). Liver is a vital organ for detoxifying chemicals of the body and play important role in the regulation of the concentration of most metabolites, mainly glucose and amino acids, it has an ability in synthesizing, souring, secreting, regulating, transforming and breaking down of several materials in the body (Akharaiyi *et al*., 2012). Serum AST and ALT activity are used as most sensitive biomarkers in diagnosis of liver diseases (Pari and Kumar, 2002). The present study was aimed to investigate the effect of acrylamide administration on thyroid and liver functions and histopathological changes in adult male rats.

2. Materials and Methods

Acrylamide of 99.9 purity from sigma chemical Co. was used in the present study.

Experimental Animals

Twenty adult healthy rats weighting (205.00±19.00) gram was used in this study. Rats were kept in the animal house for two weeks before beginning of the experiment for adaptation.

Experimental Design

Twenty adult male rats randomly divided into two groups as the following:

Group I (control): Ten adult male rats orally administered detailed water (0.4 ml/animal) by Cavage daily for 45 days. **Group II:** Ten adult male rats were orally administered acrylamide (5 mg/Kg. B.W.) dissolved in 0.4 ml distilled water by garbage daily for 45 days. At the end of the experiment ten animals of each group were anaestheized by chloroform and sacrificed. The blood samples were collected between 9:00 to 11:00 A.M. in order to minimize the diurnal variation of hormone levels by heart puncture of each rat and serum were separated for hormonal and biochemical tests and the following organs (thyroid and liver) were excised and maintained at 10 % neutral buffered formalin for histopathological study. Blood samples were collected from the heart via cardiac puncture of sacrificed animals using 5cc sterile syringe dropped in plain without anticoagulant tubes and serum samples were isolated from blood by centrifugation at 3000rpm for 15 min. and seperated in eppendroff tubes and stored at -20°C until used for hormonal and biochemical analysis.

Biochemical analysis

The basic principle of an Enzyme linked immunosorbent is to use an enzyme to detect the binding of antigen (Ag) antibody (Ab). The enzyme converts a colorless substrate to a colored product, indicating the presence of Ag: Ab binding, the estimation of thyroid stimulating hormone (TSH), total thyroxine (tT4) and tetraiodthyronine (tT3) Concentrations described by Ma *et al*. (2006). Measurement of serum tetraiodthyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction; kit was used (Monobind Inc. lake forest CA 92630, USA).

Histopathological Study

Specimens from thyroid and liver were collected and preserved in 10% neutral buffered formalin and processed to obtain paraffin sections of 6 micron thickness, then stained with hematoxylin and eosin stain (Bancroft *et al*., 1996). Then the slide is uses for histolopathological examination under light microscope.

Statistical Analyses Method

Statistical analyses were performed by the standard method. All the results were expressed as mean ± standard deviation (S.D.). The mean of the all groups compared using independent t-test. 'P' value of less than 0.05 was considered to represent statistically significant change (Abo-Allam, 2003).

3. Results

The results presented in Table - 1 revealed a significant (P<0.05) decrease in serum concentrations of TSH, T4 and T3 in ACR-treated group compared with the control group.

Table - 1: Effect ACR on serum concentrations of TSH, T4 and T3 of adult male rats (M±SD) (n=10)

Groups	TSH µIU/ml	T_4 µg/dl	T_3 ng/dl
G1 (Control)	18.85±1.45[a]	9.38±0.86[a]	2.13±1.37[a]
G2 (ACR)	8.72±1.74[b]	4.63±0.91[b]	1.03±0.55[b]
LSD	10.13	4.74	1.01

The values expressed in small letters mean significant differences in (P<0.05) levels.

The effect of ACR-treatment for 45 days on serum ALT, AST and ALP concentrations are presented in Table - 2. The results indicated that there were a significant (P<0.05) increased in serum ALT, AST and ALP concentrations in ACR-treated group compared with control.

Table – 2: Effect of ACR on serum ALT, AST and ALP in adult male rats (M±SD) (n=10)

Groups	ALT (U/l)	AST (U/l)	ALP (U/l)
G1(Control)	17.80±4.00[b]	22.00±3.41[b]	11.77±1.40[b]
G2 (ACR)	26.96±5.10[a]	38.58±4.69[a]	18.63±1.40[a]
LSD	9.16	16.58	6.85

The values expressed in small letters mean significant differences at (P<0.05) levels

Hisopathological examination: Thyroid gland - Thyroid gland of control male rats showed the normal architecture composed of thyroid follicles of different sizes lined by single layer of cuboidal thyrocytes and parafollicular cells (c-cells) as shown in Figure - 1. While histopathological section in thyroid gland of adult male rats treated with ACR for 45 days revealed that thyroid follicles of irregular shapes and sizes with vacuolated non-homogenized colloid and non clear of thyrocytes lining the follicles and parafolilicular cells. **Liver -** The section of liver in control group showing normal hypatocytes, sinusoids emptied into a clear central veins hepatocytes radiate as hepatic plates from central vein (Figure - 3) while in Figure – 4. Liver of ACR group showed clear blood accumulation in the central vein and clear flatting of hepatocytes with obvious vacuolation and absence of sinusoids imported into the central vein.

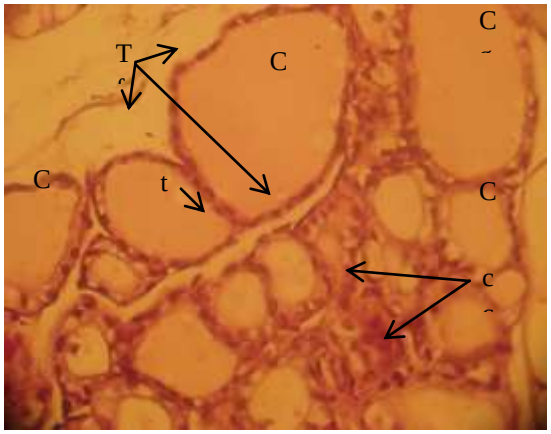

Fig. (1): histopathological section in the thyroid gland of control male rats showed normal thyroid follicles (Tf), filled with homogenized colloid (C), lined by single layer of cuboidal thyrocytes parafollicular cells (cc) (H&E stain 400X)

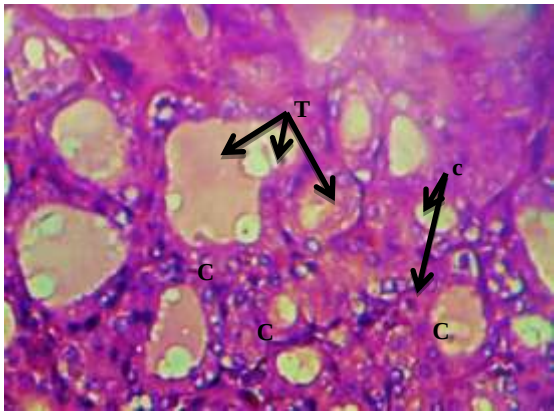

Fig. (2): Thyroid gland of ACR-treated group showing irregular size and shape of thyroid follicles(Tf) with no

clear lining thyrocytes (tc), vacuolation of colloid (c) and prafollicular cells (cc) (H&E stain 400X).

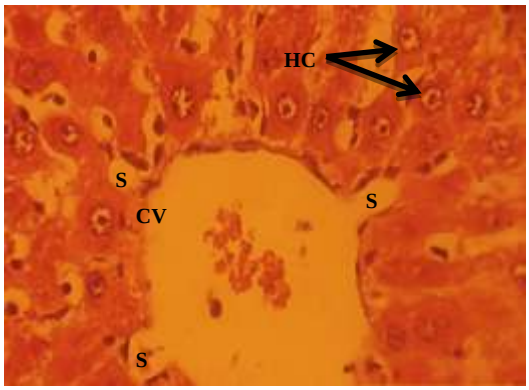

Fig.(3): Liver of control group showing normal hypatocytes(HC), sinsoids (S) emptied into a clear central vein (CV) hepatocytes radiate as hepatic plates from central vein (H& E stain 400X).

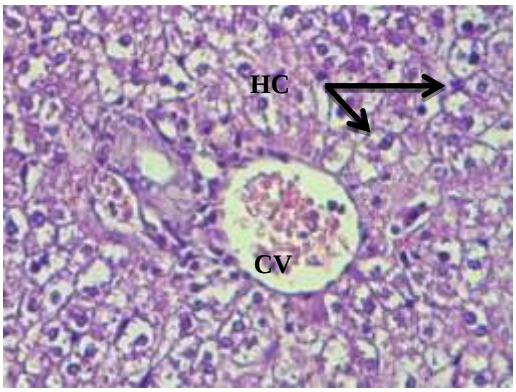

Fig. (4): Liver of ACR group showed clear blood accumulation in the central vein (CV) and clear flatting of hepatocytes (HC) with obvious vaculation and absence of sinusoids embeted into the central vein (H&E stain 400X).

4. Discussion

The present results are consistent with El-Elaimy (2006) and Manna *et al.* (2006) who all indicated that adult male rats treated with ACR resulted in a significant decrease in serum T_3 and T_4 levels compared with control. While Bower *et al.* (2008) demonstrated that T_4 level decreased only with high doses of ACR-treatment. Similarly Hamdy *et al*. (2012) showed that adult male rats treated with ACR in doses of 5, 10 and 15 mg/kg BW/day for 8 weeks cause an in serum T_3 and T_4 levels compared with controls. In contrast of the present study Khan *et al*. (1999) reported that meanling rats treated orally with ACR in doses of 2 mg and 15 mg/kg Bw/day for 2 or 7days showed a slight dose-dependent increase in plasma T_4 level and slight dose-dependent decrease in plasma TSH. Moreover Abd El-Mottaleb and Rashed (2008) reported that adult male treated with 75 and 150 mg/kg bw/day ACR for 28 days showed a significant decrease of serum T_3 and T_4 were recorded in group intoxicated with 150 mg/kg/day ACR compared with control. The reduction in thyroid hormone in the present study may be attributed to the iodine deficiency and then the thyroid gland fails to synthesize thyroid hormones and the animal suffered from hypothyroidism (Kaneko *et al*., 1997) or may be occurred as a secondary resulted due to pituitary insufficiency. The reduction of thyroid hormone levels may be resulted from increased deiodination and biliary excretion and eventually increase the rate of elimination of thyroid hormones from circulation as that occurred in pesticide toxicity (Farokhi and Taravati, 2014).

The data illustrated in Table - 2 represent the effect of ACR treatment for 45 days on serum aspartate aminotransferasr, alanine aminotranstferase and alkaline phosphatase levels. The present results are in accordance with Sawka *et al.* (2007); Sharma and Jain (2008); Tedor *et al*. (2011) and Gabr *et al*. (2010) who all found that adult male rats treated with ACR showed a significant increase in serum AST, ALT and ALP activity compared with the control group. The results in line with those of El-Bohi *et al*. (2011) who demonstrated that administration of ACR resulted in a significant increase in the activities of serum AST and ALT enzymes in adult male rats compared with the control group. The elevation of AST and ALT enzymes may result from the destruction of cellular lysosome which in turn lead to increase autolysis process. The present results are in the study made in adult albino mice by Sharma and Jain (2008) who found adult male mice treated with different doses of ACR (5, 15 and 25 mg/kg BW/dy) for two months cause a significant increase in serum AST, ALT and ALP activities

in all ACR- treated groups compared with control. The present results for the effect of ACR upon the serum liver enzymes activity also agreed with Siahkoohi *et al.* (2014) and Osman *et al.* (2016) who reported a significant increase in serum AST, ALT and ALP levels in ACR-treated adult male rats compared with control. The elevation in serum AST, ALT and ALP activities may be resulted from the increase permeability of hepatocyte cell membrane which may occur due to the toxic effect of ACR which cause cellular damage and in turn leakage of the enzymes outside the cellular structure to extracellular fluid. The elevation of serum AST, ALT and ALP activities may occurred due to the bipolar nature of ACR the CH_2=CH-part may undergo hydrophobic interaction and the $–CONH_2$ part can form hydrogen bonds with the cell component, this property may enhance its ability to alter the structure of cell membarne structure and make the paranchymal cell membrane of the liver more permeable, ther by causing the active retention of enzymes and making them appear first in the extracellular space and then in the blood (Chinoy and Memon, 2001).

Histopathological studies, **Thyroid gland** - The microscopic examination of the thyroid gland of ACR-treated rats showed avariable pathological changes which in consistent with that obtained by Khan (1999) who showed a significant reduction in the colloid area and a significant increase in the hight of thyrocytes in ACR-treated rats.with (2 and 5 mg/kg BW/day) for 2 and 7 days. Similarly polychlorinated biphenyls treatment produces striking hypertrophy and hyperplasia of thyroid folliclar cells, along with a significant reduction in serum T_4 level in rats (Capen & Martin, 1989). However Osman *et al*. (2016) reported that some follicles were dilated and lined with flattened epithelium with colloid in their lumen and other follicles were reduced in size with few colloidal materials. This may explain the decrease in T_3 and T_4 in ACR treated male rats. In contrast of the results of present study Imai *et al.* (2008) did not observe any hisopathological changes in thyroid of males and females treated with ACR in drinking water at (1.0, 2.1 and 4.4 mg/kg BW/ day) in males and 1.2, 2.5 or 4.9 mg/kg BW/day) in females for 12 weeks.

Liver - A microscopic examination of the liver section of male treated with ACR showed different pathological changes as show in figure (4) these results are accorded with Abd El-Mottaleb and Rashed (2008) and Allam *et al.* (2010) who reported that the liver of rats treated with low dose of ACR showed vacuolar degenerative changes of hepatocyes and blood vessel congestion. The significant increase in the levels of serum liver enzymes resulted from these pathological changes in the liver. Rahangadale *et al*. (2012) also revealed the liver of ACR-treated group appeared congestion and Kupper cell proliferation. While Khan *et al*. (2011) showed disruption of hepatic cords, infiltration of inflammatory cells in the central vein, vacuolation of hepatocytes and necrosis. On other hand liver rats treated with ACR showed frequent necrosis and bleeding indicated hypertrophy of nuclei, pyknotic nuclei, proliferation of sinusoidal bile duct and hemorrhage (Vasundhara, 2005; Nagao *et al*, 2007). The vacuilation of the hepatocytes cytoplasm may be occurring due to progressive ischemia, hypoxia and accumulation of lipid in the hepatocytes (Abdul-Hamid and Moustafa, 2005 and Moustafa and Abdul-Hamid, 2007).

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