

Full Length Research Paper

Hazardous effects of acrylamide on immature male and female rats

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Acrylamide (ACR) is an industrial chemical which induces neurotoxic effects in experimental animals and humans. The present study was carried out to investigate the hematological, biochemical, neurological and histopathological effects of ACR on immature male and female rats. Animals were divided into 2 main groups; immature male group and immature female group and all rats were treated for 28 consecutive days. Each main group subsequently was divided into 2 subgroups: (I) Untreated control group that received a daily oral administration of distilled water and (II) ACR treated rats which received a daily oral administration of ACR (15 mg/kg/body weight). The results obtained indicate that ACR administration induced some behavioral disorders in the movement of immature male and female rats as well as loss of body weight. ACR induced a significant decrease in hemoglobin (Hb), erythrocytes (RBCs), hematocrit (HCT) and lymphocyte levels of young female rats. ACR significantly increased serum glucose, total cholesterol and triglycerides concentrations of both immature male and female rats. While, significant increase in the total urea concentration was noticed only in the immature male rats following ACR administration. Moreover, ACR induced marked increase in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the immature male and female rats. On the other hand, the activities of serum alkaline phosphatase (ALP) and acetylcholinesterase (AChE) were significantly decreased in both treated groups. ACR caused a significant increase in norepinephrin (NE), glutamate, aspartate and taurine, while it reduced dopamine (DA) and serotonin (5-HT) levels. In conclusion, the present study showed that, ACR induced hazardous effects on immature male and female rats. So, we recommended that children must avoid fast or junk foods.

Key words: Acrylamide, hematological, biochemical, neurological, histopathological, immature.

INTRODUCTION

Acrylamide (ACR) is a reactive, highly water-soluble vinyl monomer. It is an industrial chemical used in the manufacture of polyacrylamides that are common to personal care and grooming products, such as lotions, cosmetics and deodorants (Exon, 2006). In addition to Industrial and laboratory uses, it has been reported that the formation of ACR is associated with high-temperature (higher than 200°C) cooking process in certain carbohydrate-rich foods, especially when asparagines

react with sugar (Parzefall, 2008). Therefore, the general population can be exposed to ACR through their diets. ACR has been reported to be neurotoxic (Lehning et al., 2003; Seale et al., 2011), toxic to the reproductive system (Favor and Shelby, 2005; Ma et al., 2011) and carcinogenic in experimental animals (Hogervorst et al., 2010). Studies on experimental animal models showed that ACR exposure results in hind-limb foot splay, ataxia and skeletal muscle weakness (Shukla et al., 2002). Similar symptoms, including weight loss, have also been documented in the neurotoxic syndrome in humans (LoPachin, 2004).

The neurotoxicological alterations that may occur with

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developmental exposure to ACR, including paraesthesias in fingers, coldness and weakness of hands, numbness in lower limbs, drowsiness, hallucinations, ataxia, convulsion, diffused damage to different sections of the nervous system, lysis in the cerebellum neurons and tibial nerve degeneration (Bachmann et al., 1992; Gold et al., 2004).

Orally consumed ACR is absorbed into the circulation, then distributed to various organs, and reacts with DNA, neurons, hemoglobin, and essential enzymes causing several toxic effects (Baum et al., 2008; Rayburn and Friedman, 2010). ACR is not genotoxic by itself but becomes activated to its primary epoxide genotoxic metabolite glycidamide (GA) via epoxidation (Baum et al., 2008), by CYP2E1 which leads to the formation of GA-DNA and hemoglobin adducts (Ghanayem et al., 2005).

ACR is known to cause toxicity in the experimental animals and humans, but its effect on the embryonic and postnatal development is relatively less understood (Allam et al., 2010). It is anticipated that children will generally have intakes that are two to three times those of adults when expressed on a body-weight basis. Though exposure to acrylamide is inevitable, it is necessary to protect infants and children from high exposure. So, the present study focuses on the several adverse health effects of ACR on immature male and female rats.

MATERIALS AND METHODS

Chemical

Acrylamide monomer dry crystals (C_3H_5NO , > 99% purity), CAT No. 150256 79-061 purchased from MP Biomedicals, LLC. France was used in the present study.

Experimental animals

The experimental animals used in this study were the weaned male and female rats of 21 days old (*Rattus norvegicus*) weighing 50 ± 5 g. The animals were obtained from the National Research Center (NRC, Dokki, Giza). Animals were housed under normal environmental conditions of temperature and humidity. They were kept under the normal light-dark rhythm. Food and water were provided *ad libitum*. All experiments were carried out accordance with research protocols established by the animal care committee of the National Research Center, Egypt.

Experimental design

After 1 week of acclimation, male and female immature rats were divided into two main groups; Male rats and female rats (12 rats for each). Each main group are subsequently divided into two subgroups (6 rats/subgroup). Subgroup I was administered distilled water for 28 days and subgroup II was administered ACR (15 mg/kg. body weight) for 28 days.

All animals were sacrificed on the 29th day of treatment after fasted over night; blood was collected in EDTA containing tubes and centrifuged. Liver, kidney, brain, testes and ovary were

removed for further histopathological examination.

Serum preparation

Blood samples collected in centrifuge tubes were centrifuged at 3000 rpm for 20 min. Serum was stored at $-20^\circ C$ until used for biochemical assays.

Estimation of blood parameters

Haemoglobin (Hb) concentration, red blood cell (RBC) count, hematocrit (HCT), white blood cell (WBC) count, lymphocyte, monocyte, neutrophil, eosinophil, and basophil cells were estimated using a semiautomatic haematological analyzer (SWELAB IEO Model). The auto counter utilized 20 μ l of blood in 16 ml of a commercially prepared diluent. The machine's ability to count cells was based on the principle of electronic impedance.

Assessment of liver functions

The appropriate kits (Biodiagnostic kits) were used for the determination of serum total protein according to Lowry et al. (1951), aminotransferase activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to Reitman and Frankel (1957) and alkaline phosphatase (ALP) activity according to Young et al. (1975).

Assessment of renal functions

Serum samples were assayed for creatinine, uric acid, urea and blood urea nitrogen (BUN) by using standard kits (Biodiagnostic kits). Creatinine was detected by the method of Houot (1985); uric acid was detected by the method of Fossati et al. (1980) and urea and BUN were estimated by Patton and Crouch (1977) method.

Assessment of glucose and lipid profile

Serum samples were assayed by using standard kits (Biodiagnostic kits). Serum glucose level was detected by (Trinder, 1969); serum total lipid was measured by Knight et al.'s (1972) method, triglycerides was detected according to Fossati and Prencipe (1982) study; total cholesterol was determined according to Allain et al. (1974) study. LDL-cholesterol was measured according to Wieland and Seidel (1983). HDL-cholesterol was determined by the method of Lopez et al. (1977).

Assessment of acetylcholinesterase activity:

Acetylcholinesterase activity was estimated in serum using acetylthiocholine iodide as a substrate using modified Ellman (1978) method.

Brain tissue preparation

Brain was divided into two halves; the first half was used for determination of neurotransmitters (Free amino acid and monoamine), while the second half was used for histopathological examination.

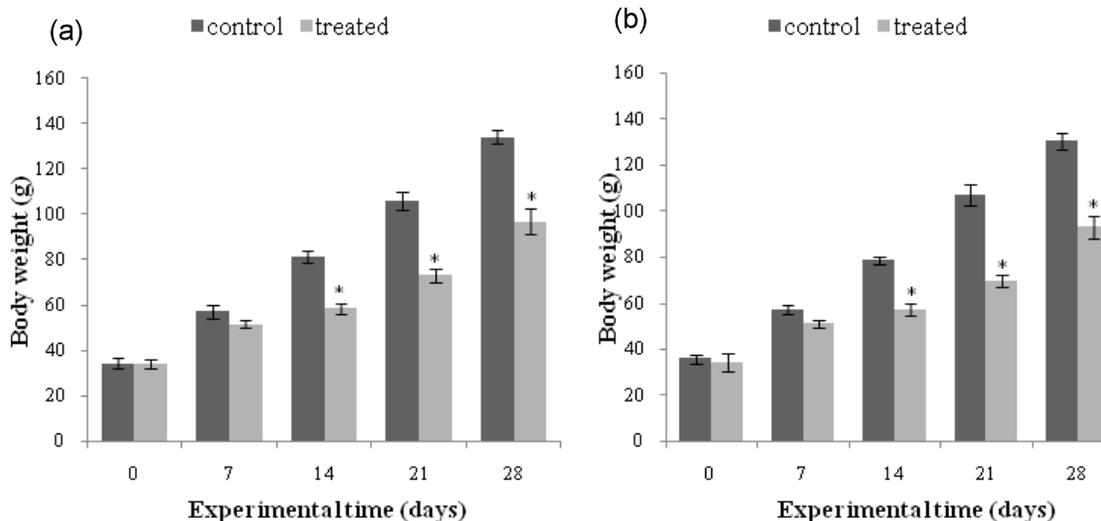


Figure 1. Effect of daily administration of acrylamide 15 mg /kg b. wt., on body weight of immature male and female albino rats after 28 days;(a) immature male; (b) immature female.

Preparation of brain tissues for neurotransmitters determination

The first step in the determination of amino acids by HPLC method involved weighing and homogenization of the brain tissue in 1/10 weight/volume of 75% aqueous HPLC grade methanol. The homogenate was spun at 4000 r.p.m. for 10 min and the supernatant was divided into two halves; the first was dried using vacuum (70 millipore) at room temperature, whereas the second half was used for monoamine determination by HPLC according to the method described in Pagel et al. (2000).

Preparation of tissues for histopathological examination

The fixed liver, brain, kidneys, testes and ovaries were embedded with paraffin blocks, and microscopic specimens were sliced, then subjected to hematoxylin and eosin stain according to Bancroft et al. (1996) for histopathological examinations through the light microscope.

Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS software for Windows. When the ANOVA test yielded statistical differences ($P < 0.05$), post hoc LSD was carried out to estimate the significant differences between rats administered ACR and their corresponding control. All data were expressed as mean \pm S.E.

RESULTS

Effect of acrylamide (ACR) on body weight

In the present study, ACR administration to immature male and female rats for 28 consecutive days induced over signs of peripheral neuropathy, such as weakness,

ataxia and dragging of hind limbs in rats at the end of the experiment. The effect of daily administration of ACR 15 mg /kg b. wt. on body weight of immature male and female albino rats after 28 days are presented in Figure 1. The results showed that ACR induced significant decrease ($P < 0.05$) in body weight of immature male and female rats as compared to their control groups.

Effect of acrylamide (ACR) on some hematological parameters

As shown in Table 1, administration of ACR for 28 days caused a significant ($P < 0.05$) decrease in hemoglobin (Hb), red blood cells (RBCs), hematocrit (HCT) and lymphocyte of young female rats as compared to their corresponding controls. While, significant decreases ($P < 0.05$) in the white blood cells (WBCs), and neutrophil numbers were recorded in both immature male and female rats.

ACR intoxication caused a significant decrease in neutrophil count of male rats, but it caused a significant ($P < 0.05$) increase in neutrophil count of female rats as compared to control groups.

Effect of acrylamide (ACR) on some biochemical parameters

The data observed in Table 2 indicate that ACR administration induced a significant ($P < 0.05$) increase in serum glucose concentration of young male and female rats as compared to control group. On the other hand, ACR intoxication induced a significant increase in urea

Table 1. Effect of oral administration of ACR (15 mg/kg b.wt.) for 28 days on the levels of blood parameters (Hb, RBCs, WBCs, HCT and differential leucocytes count) of young male and female albino rats.

Group Parameter	Male		Female	
	Control	Treated	Control	Treated
Hb (g/dl)	14.67±0.24	13.90±0.30	14.92 ± 0.32	13.15±0.59*
RBCs (10 ⁶ /mm ³)	7.22±0.07	6.74±0.15	7.64±0.20	6.33±0.31*
HCT (%)	38.20±0.80	37.57±0.35	38.35±0.88	34.53±0.70*
WBCs (10 ³ /mm ³)	10.42±0.71	5.47±0.29*	11.69±0.49	9.53±0.04*
Lymphocyte (%)	72.48±1.05	71.57±1.72	80.00±1.55	74.17±1.33*
Monocyte (%)	4.83±0.31	5.00±0.26	5.00±0.37	5.33±0.21
Neutrophil (%)	14.83±0.60	12.5±0.76*	8.33±0.61	15.77±1.27*
Eosinophil (%)	2.50±0.22	2.50±0.22	2.67±0.33	2.50±0.22
Basophile (%)	2.00±0.25	1.80±0.17	2.00±0.25	1.5±0.22

Values are given as mean ± SE for 6 rats in each group; *, significant (P<0.05) as compared with the control group.

Table 2. Effect of oral administration of ACR (15 mg/kg b.wt.) for 28 days on the levels of glucose, total protein, urea, uric acid, creatinine, total lipid, triglycerides, cholesterol, LDL-cholesterol, HDL-cholesterol of young male and female albino rats.

Group Parameter	Male		Female	
	Control	Treated	Control	Treated
Glucose (mg/dl)	67.47±1.66	88.57±2.79*	66.08±3.03	83.43±1.81*
Total protein (g/dl)	5.59±0.18	5.15±0.08	6.02±0.14	5.60±0.11
Urea (mg/dl)	84.30±4.41	116.67±5.27*	40.51±1.90	50.46±2.14
Uric acid (mg/dl)	1.80±0.28	1.72±0.09	2.33±0.10	2.12±0.17
Creatinine (mg/dl)	0.58±0.02	0.95±0.10	0.70±0.03	0.67±0.02
Total lipid (g/dl)	1.71±0.20	1.84±0.10	1.64±0.22	3.50 ±0.28*
Triglycerides (mg/dl)	46.67±2.20	33.12±1.75*	46.73±2.68	36.63±1.46*
Cholesterol (mg/dl)	63.28±2.08	76.08±4.43*	61.59±4.60	75.91±0.64*
LDL-cholesterol (mg/dl)	77.91±2.35	84.78±4.26	71.02±3.35	68.11±1.21
HDL-cholesterol (mg/dl)	81.30±2.96	71.10±3.10	80.26±4.87	85.35±2.70

Values are given as mean ± SE for 6 rats in each group; *, significant (P<0.05) as compared with the control group.

Table 3. Effect of oral administration of ACR (15 mg/kg b.wt.) for 28 days on the activities of ALP, ALAT, ASAT, AChE of young male and female albino rats.

Group Parameter	Male		Female	
	Control	Treated	Control	Treated
ALP (IU/L)	176.51±6.65	152.43±2.47*	154.94±5.94	119.76±6.09*
ALAT (U/ml)	48.97±0.70	53.21±0.70*	47.71±1.05	51.29±0.57
ASAT (U/ml)	81.89±1.35	88.87±1.21	82.15±1.42	87.51±1.61
AChE (U/L)	5.79±0.28	4.03±0.33*	5.44±0.47	4.29±0.55*

Values are given as mean ± SE for 6 rats in each group; *, significant (P<0.05) as compared with the control group.

concentrations (P<0.05) of young male rats compared to control group. Also, treatment with ACR caused a significant (P<0.05) increase in serum total lipid of female rats versus control values. On the other hand, there were a significant (P<0.05) decrease in triglycerides (TG) of young male and female rats as compared to the control

groups. Also, ACR administration caused a significant increase (P<0.05) in serum total cholesterol of young male and female albino rats as compared to control group.

Data presented in the Table 3 showed a significant (P<0.05) inhibition in the activity of alkaline phosphatase

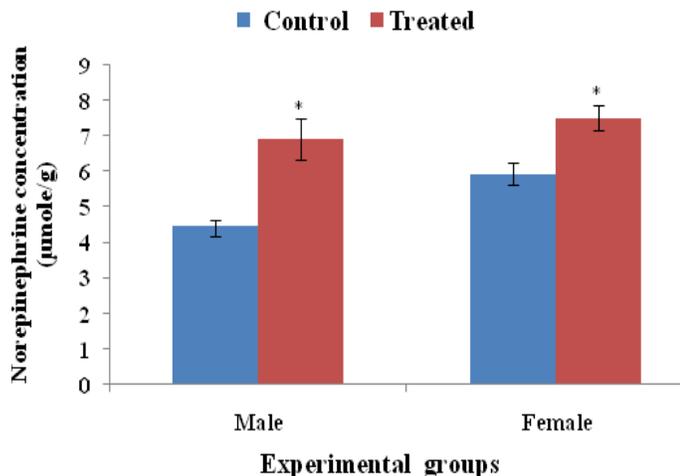


Figure 2. Effect of acrylamide (ACR) administration for 28 days on noradrenaline of young male and female rats.

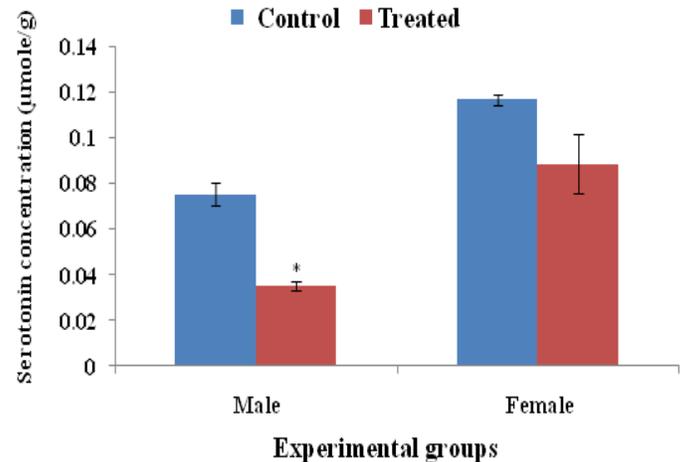


Figure 4. Effect of acrylamide (ACR) administration for 28 days on serotonin levels of young male and female rats.

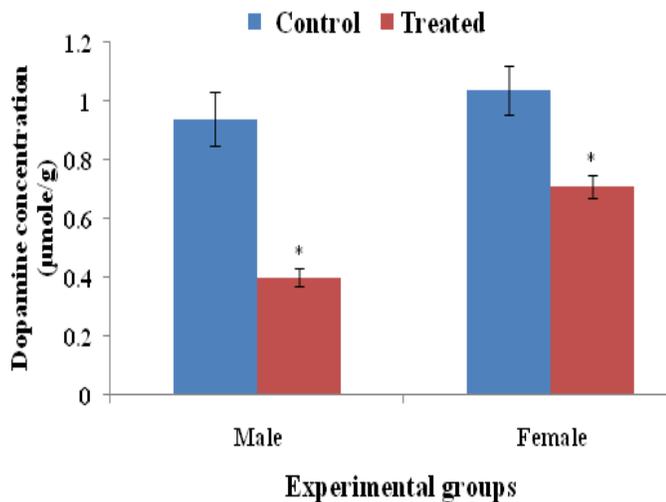


Figure 3. Effect of acrylamide (ACR) administration for 28 days on dopamine levels of young male and female rats.

(ALP) in both young male and female rats following ACR treatment. On the other hand, ALAT activity was increased only in the immature male rats as compared to the control group. Acetylcholinesterase (AChE) activity was significantly ($P<0.01$) inhibited in the serum of young male and female rats.

Effect of acrylamide (ACR) on some neurological parameters

As shown in Figure 2, ACR administration for 28 consecutive days induced a significant ($P<0.05$) elevation in norepinephrine (NE) levels in whole brain of immature male and female rats compared to control groups. Figure 3 indicate that ACR induced significant ($P<0.05$)

decrease in Dopamine levels in brain of young male and female rats compared to control groups.

ACR administration for 28 days induced a significant decrease ($P<0.05$) in serotonin levels of the brain of both young male and female albino rats, but this decrease was strongly in immature male rats (Figure 4).

As shown in Table 4, ACR administration for 28 consecutive days induced statistical significant ($P<0.05$) increase in glutamate, and aspartate levels of immature male and female albino rats.

After 28 days of ACR treatment, glycine and alanine content exhibited significant ($P<0.05$) increase in whole brain of young male and female rats, compared with that of the control groups (Table 4). While, there was a significant ($P<0.05$) decrease in whole brain gamma-aminobutyric acid (GABA) concentration for 7 days of young female rats, the whole brain concentration of taurine exhibited a significant ($P<0.05$) increase after 28 days of ACR intoxication.

Histopathological effect of acrylamide administration on immature male and female albino rats following 28 days

Microscopic examination of the control immature male rat liver shows the normal histological structure of the central vein and portal area with the surrounding hepatocytes (Figure 5). Following 28 days of acrylamide treatment, diffuse kupffer cells proliferation was detected in between the degenerated hepatocytes (Figure 6).

Histopathological examination of the kidney tissues of the control immature male rats shows normal histological structure of the glomeruli and tubules in the cortex and medulla (Figure 7). As compared to the control, the most remarkable effects of the continuous administration of acrylamide for 28 days showed congestion in the cortical blood vessels associated with focal fibroblastic cells

Table 4. Effect of oral administration of ACR (15 mg/kg b.wt.) for 28 days on the levels of free amino acids (glutamate, aspartate, GABA, glycine, alanine and taurine) of young male and female albino rats.

Parameter	Male		Female	
	Control	Treated	Control	Treated
Aspartate	3.73±0.12	6.22±0.25*	3.53±0.14	6.54±0.60*
Glutamate	10.17±0.08	13.39±0.67*	9.39±0.54	16.02±0.29*
Glycine	3.21±0.38	6.77±0.52*	3.51±0.48	4.85±0.51*
GABA	2.32±0.15	2.25±0.06	3.03±0.34	2.76±0.41
Taurine	4.36±0.21	8.41±0.52*	4.24±0.11	4.55±0.15
Alanine	1.39±0.09	1.82±0.35*	1.10±0.04	1.83±0.25*

Values are given as mean ± SE for 6 rats in each group. *, significant ($P < 0.05$) as compared with the control group.

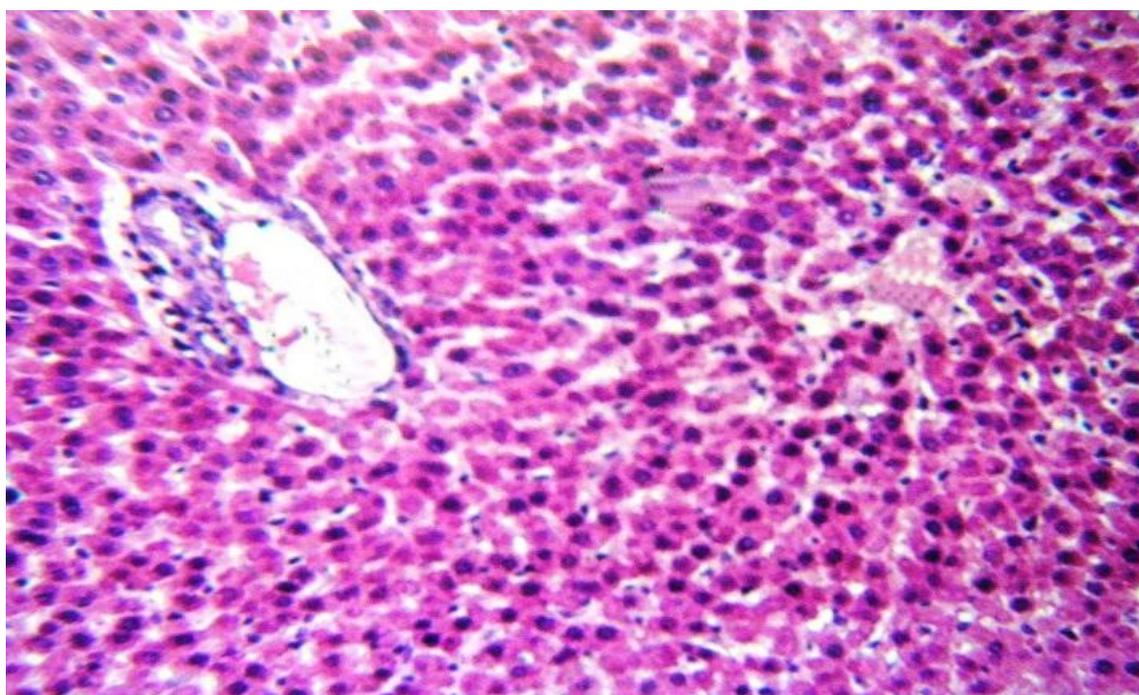


Figure 5. Liver section of control immature male rat showing the normal histological structure of the central vein (c), portal vein in portal area and surrounding hepatocytes (h).

proliferation in between the tubules (Figure 8).

Brain section of control immature male rats showed normal histological structure of the cerebral cortex and cerebellum (Figure 9). Concerning the histopathological examination of the brain tissues of acrylamide-treated rats for 28 days, the obtained results showed vacuolization in cerebrum (Figure 10).

Microscopic examination of the testes of the control immature male rats showed normal histological structure (Figure 11). Post twenty eight days of acrylamide administration, Pyknosis in the nuclei of some spermatogonial cells were noticed (Figure 12) Investigation of histopathological examination of cross section of the liver tissue of the control female immature

rat showed normal histological structure of the central vein and surrounding hepatocytes (Figure 13). After 28 days of acrylamide administration, the histopathological data showed hypertrophy in the proliferated kupffer cells and apoptosis in some hepatocytes (Figure 14).

Figure 15 shows normal histological structure of the glomeruli and tubules in the cortex and medulla of the kidney tissues of immature female rats. Histopathological examination of the kidney following acrylamide intoxication for 28 days showed congestion in the cortical blood vessels (Figure 16).

Microscopic examination of the brain sections of the immature female rats showed normal histological structure of the cerebral cortex and cerebellum

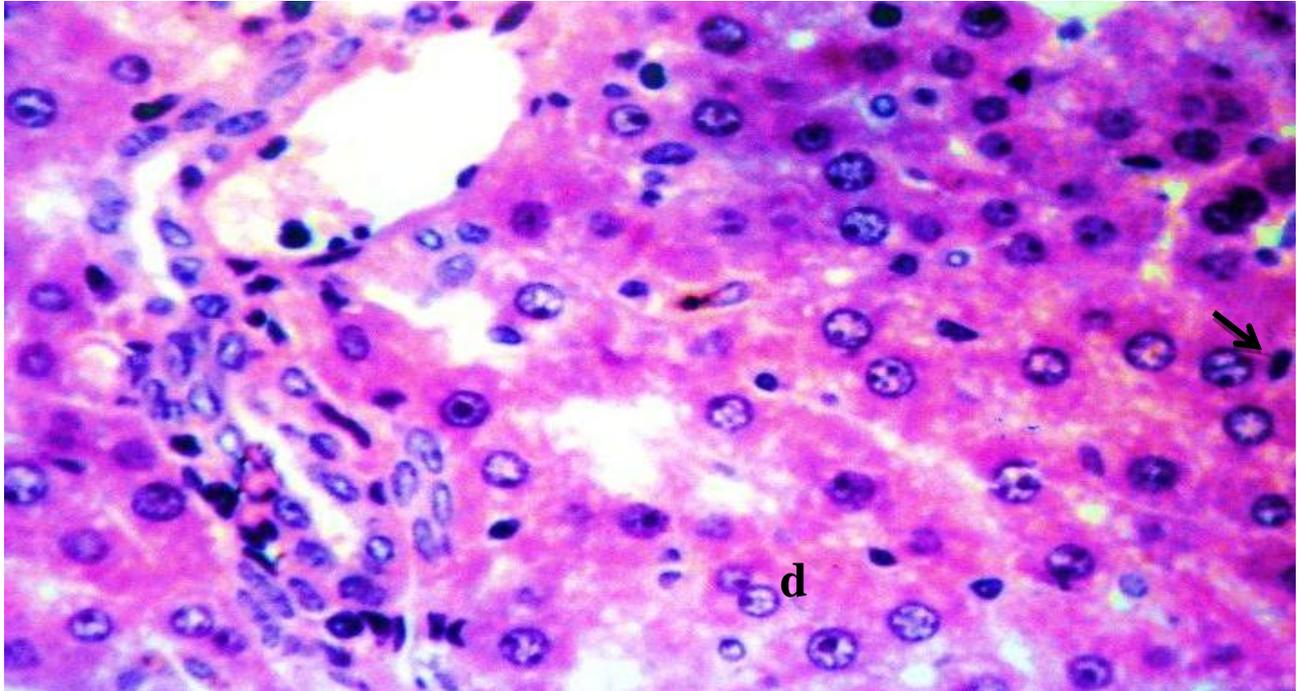


Figure 6. Liver section of immature male rat administered acrylamide for 28 days showing diffuse kupffer cells proliferation (arrow) in between degeneration of the hepatocytes (d).

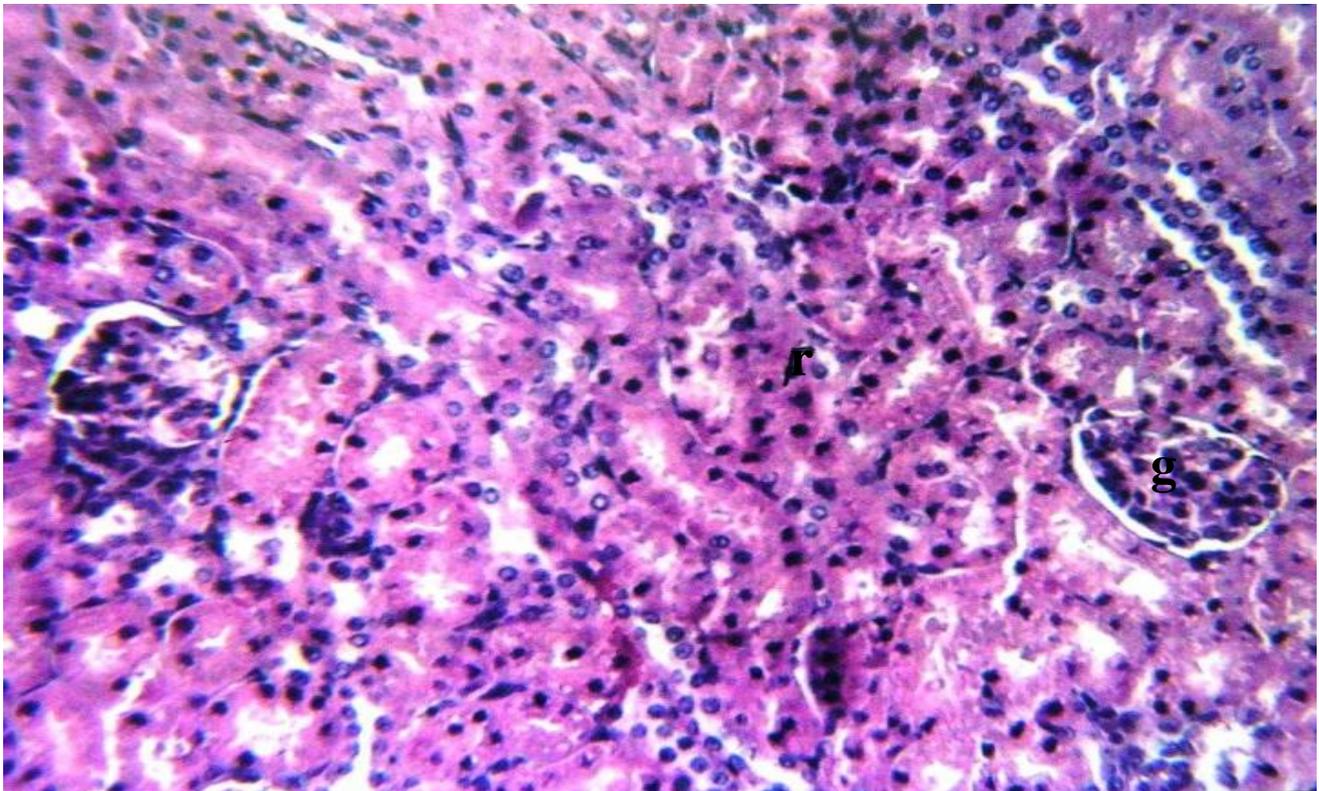


Figure 7. Kidney section of control immature male rat showing the normal histological structure of the glomeruli (g) and surrounding tubules (r) in the cortex.

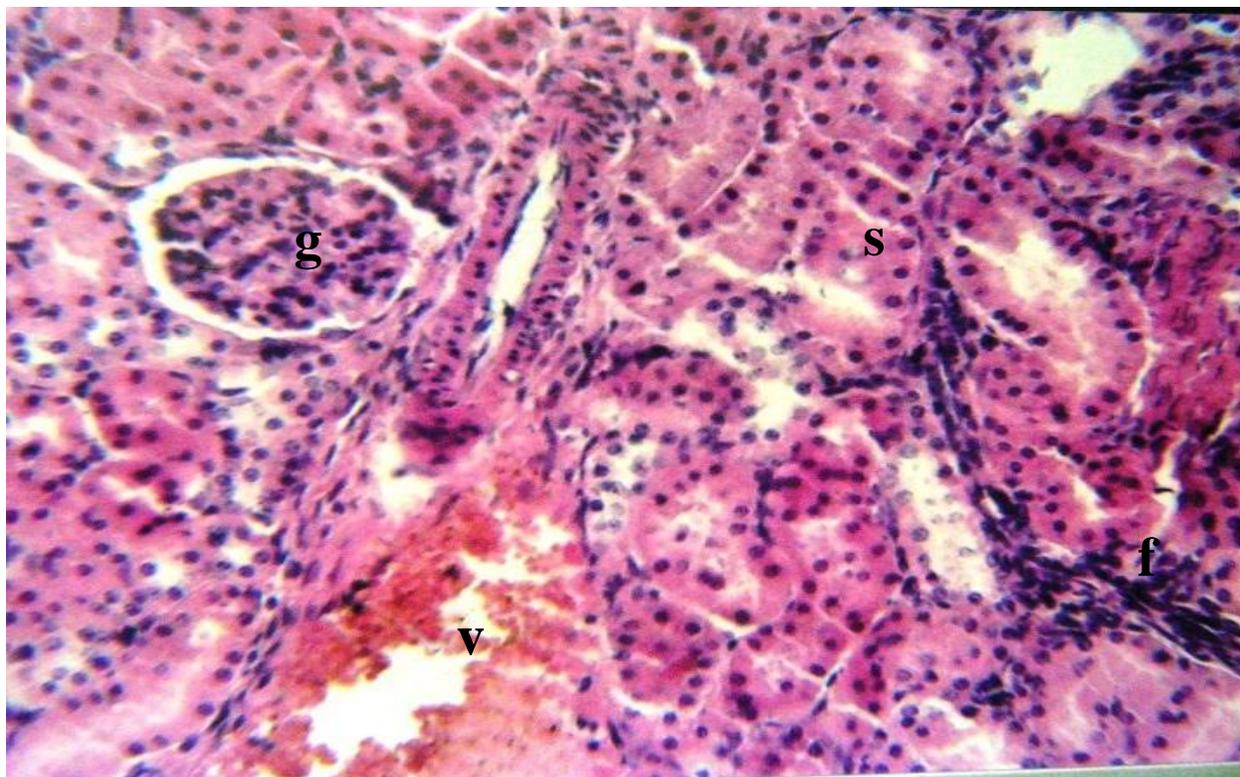


Figure 8. kidney section of immature male rat administered acrylamide for 28 days showing swelling in the epithelial cells of the tubules (s) with focal fibrosis (f) in between and congestion in blood vessels (v).

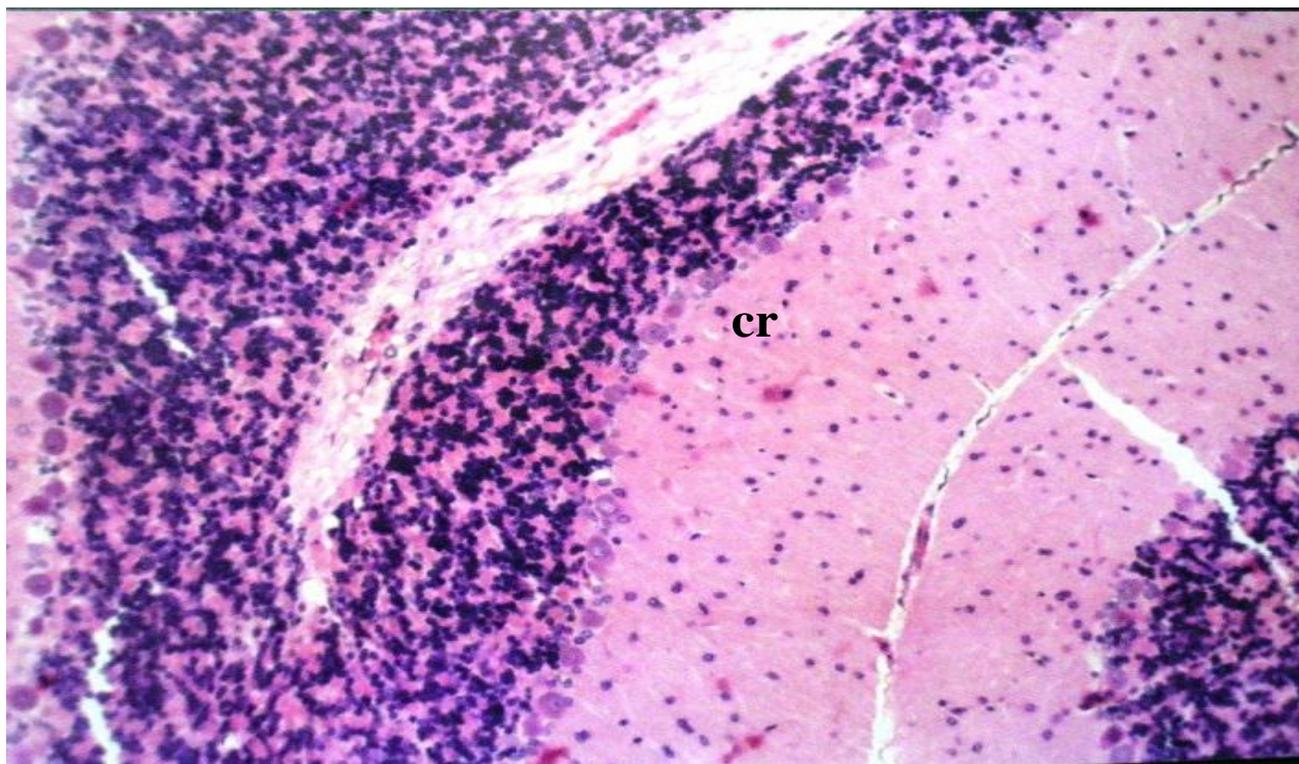


Figure 9. Brain section of control immature male rat showing the normal histological structure of the cerebellum (cr).

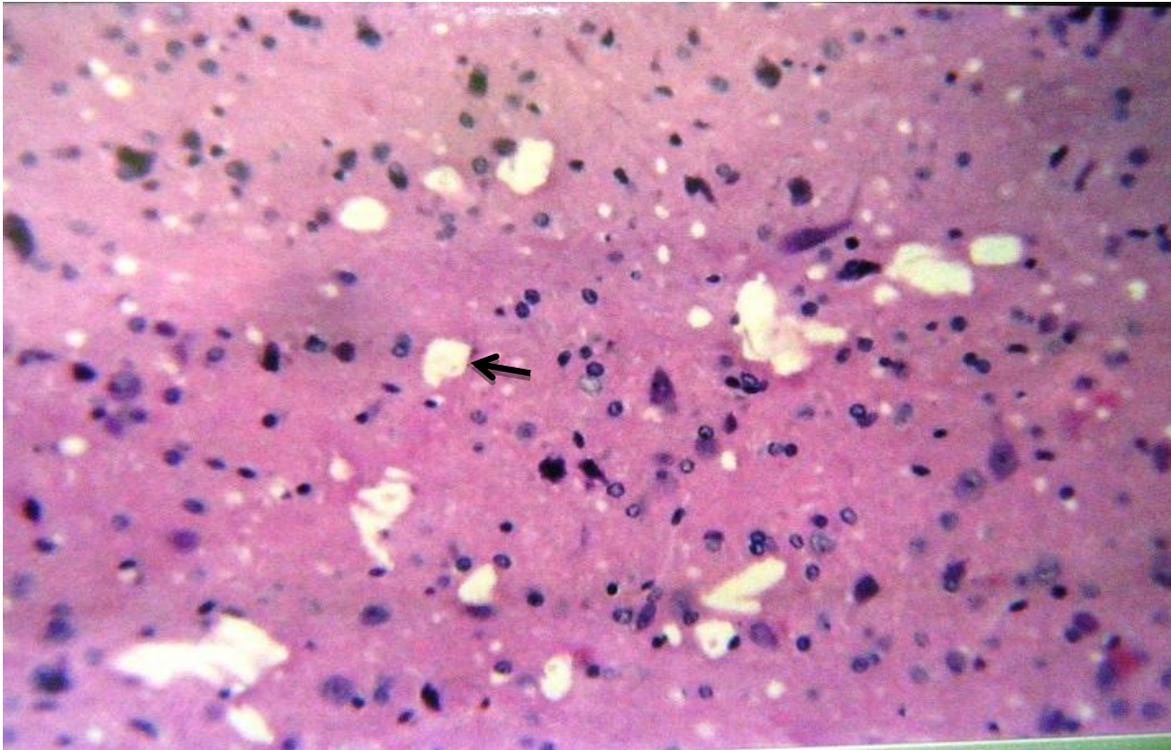


Figure 10. Brain section of immature male rat administered acrylamide for 28 days showing vacuolation in the cerebrum (arrow).

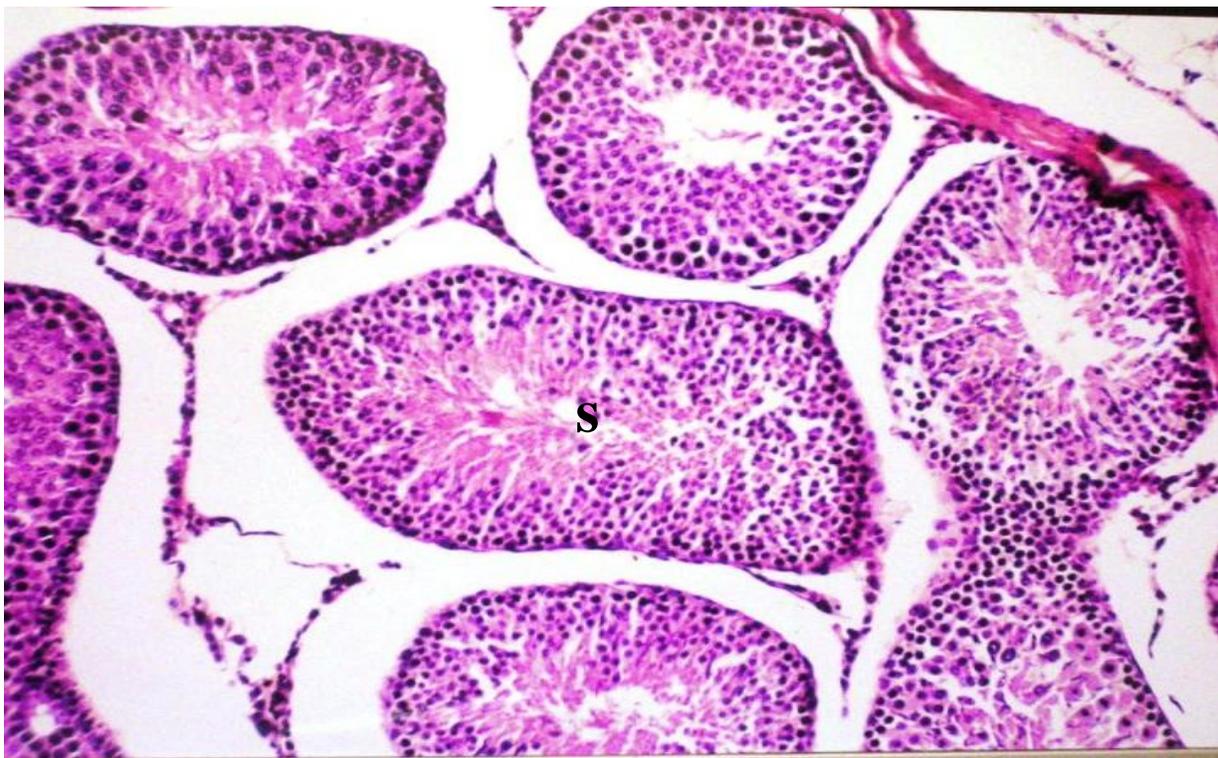


Figure 11. Testes section of control immature rat showing the normal histological structure of the mature active seminiferous tubules with complete spermatogenic series (s).

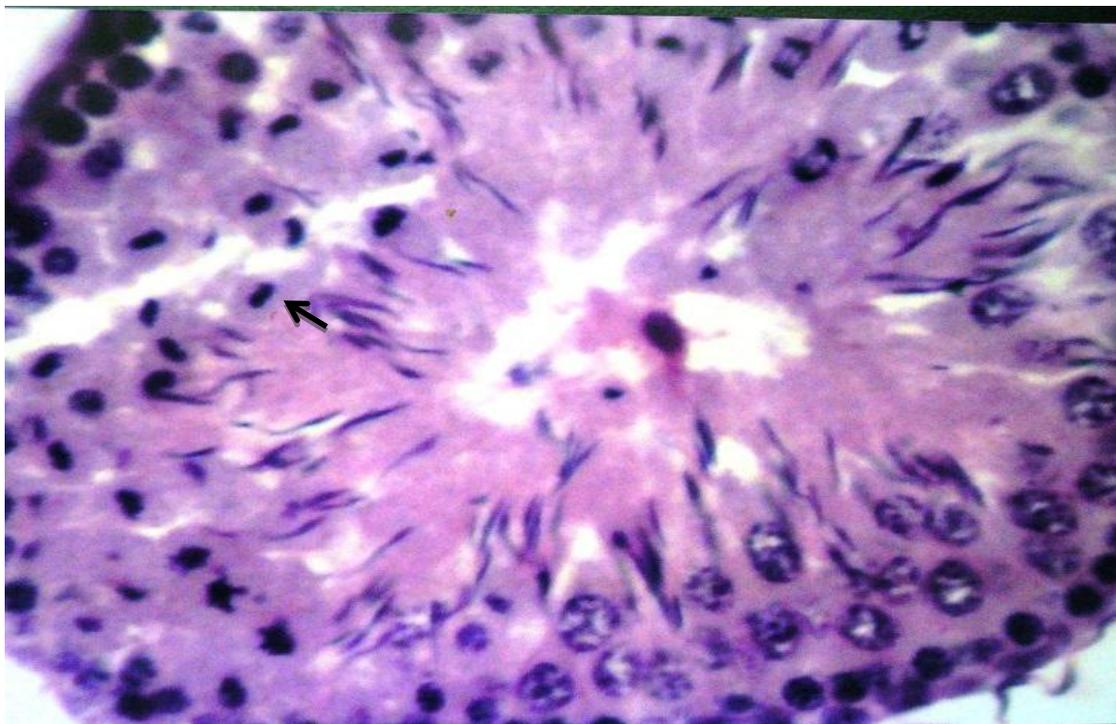


Figure 12. Testes section of immature rat showing pyknosis in the nuclei of some the seminiferous spermatogonial cells (arrow).



Figure 13. Liver section of control immature female rat showing the normal histological structure of the central vein (c) and surrounding hepatocytes (h).

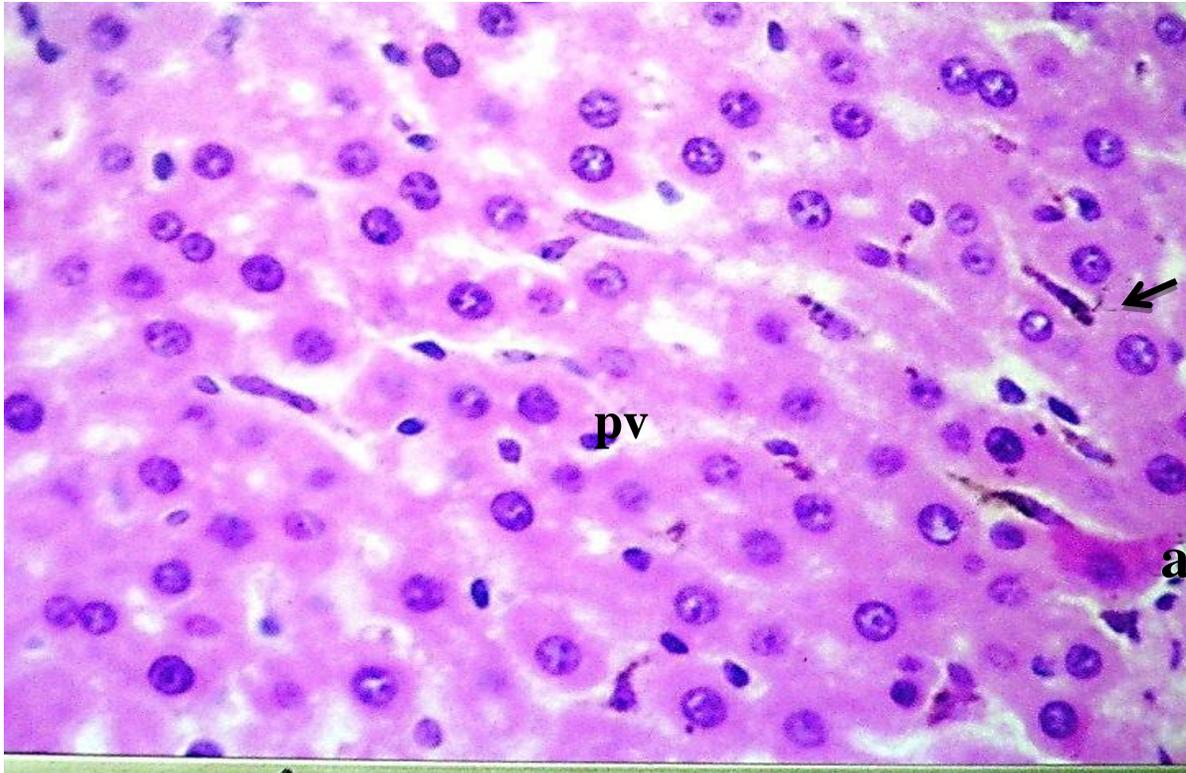


Figure 14. Liver section of immature female rat administered acrylamide for 28 days showing proliferation and hypertrophy of kupffer cells (arrow) with apoptosis in some individual hepatocytes (a).

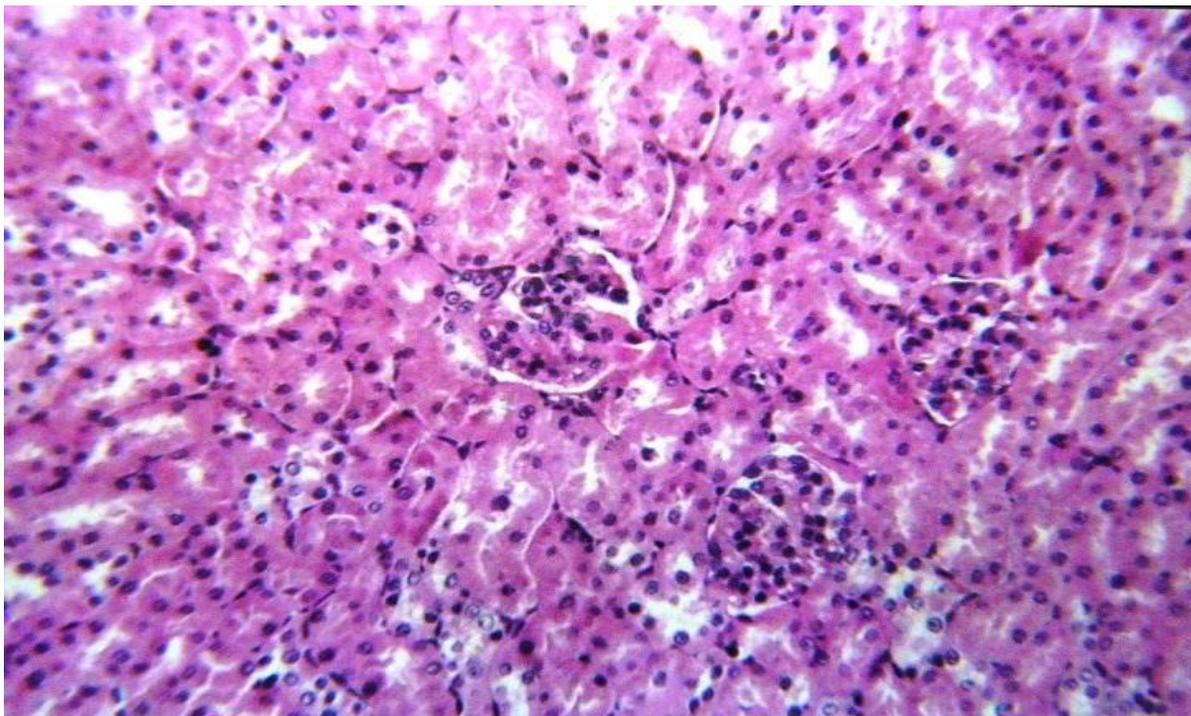


Figure 15. Kidney section of control immature female rat showing the normal histological structure of the glomeruli (g) and surrounding tubules (r) in the cortex.

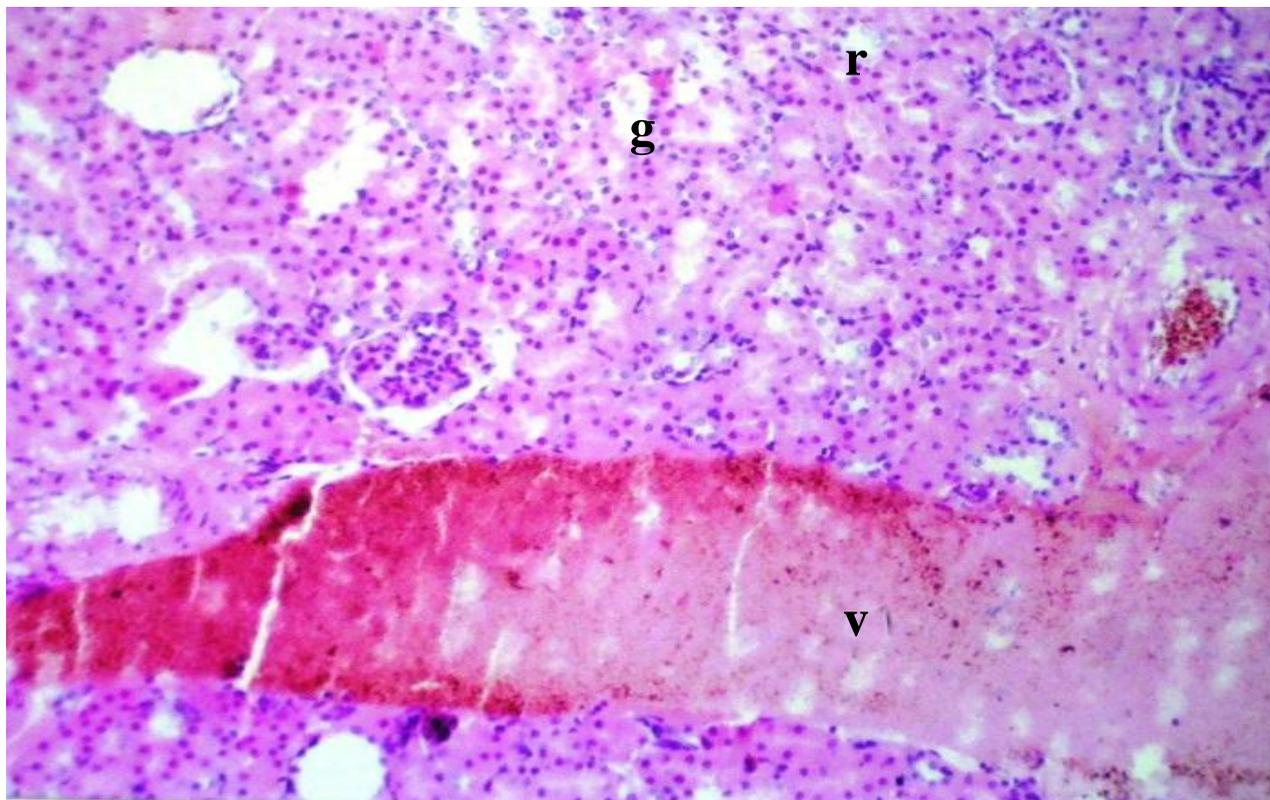


Figure 16. Kidney section of immature female rat administered acrylamide for 28 days showing dilatation and congestion in the cortical blood vessels (v).

(Figure 17). The examined sections indicated that after 28 consecutive days of acrylamide administration, vacuolization in the cerebellum was noticed (Figure 18). In the inspection of sections obtained from control and acrylamide treated immature female rats for 28 days, the results showed that acrylamide had no effect on the ovary tissue (Figures 19 and 20), respectively.

DISCUSSION

Acrylamide, one of the major environmental public health problems, results from its increased accumulation in the process of cooking food materials (Zhang and XZhang, 2007). Acrylamide (ACR) toxicity on human and experimental animals was well documented in a series of reports since the Swedish food administration alarm in 2002 (LoPachin, 2004; Park et al., 2010; Raju et al. 2011). Acrylamide is a small organic molecule with very high water solubility. These properties facilitate its rapid absorption and distribution through body (Mannaa et al., 2006).

In recent years, attention has been focused on the role of biotransformation of chemicals to highly reactive metabolites that initiate cellular toxicity. Many compounds, including clinically useful drugs can cause

cellular damage through metabolic activation of the chemicals to highly reactive compounds. The metabolism of acrylamide can explain possible harmful effects of this substance, especially in relation to carcinogenicity and mutagenicity. Hereby, the most important pathogenic pathway is the oxidative biotransformation of ACR by cytochrome P450 2E1 (CYP2E1) (Sumner et al., 1999). The resulting metabolite is an epoxide derivative, that is, glycidamide, which is more reactive toward DNA and proteins than the parent compound ACR (Dearfield et al., 1995; Besaratinia and Pfeifer, 2007). Majority of ACR is conjugated with glutathione while a lesser amount is activated via glycidamide (Parzefall, 2008).

In the current study, the administered dose of ACR was high as compared with the estimated one in the cooked food which is as high as 70 µg per day (Tareke et al., 2002). However, to clarify the effect of ACR on some physiological, biochemical parameters as well as histopathological and neurological effects in the present study, the ACR was administered to immature male and female albino rats at 15 mg/kg body weight.

In conjunction with the reports of LoPachine (2004) and El-Bohi et al. (2011), data from the present investigation reflects hind limb dysfunction and gait following ACR administration. In agreement with our results, Shukla et al. (2002) reported hind limb paralysis in 58% of the ACR

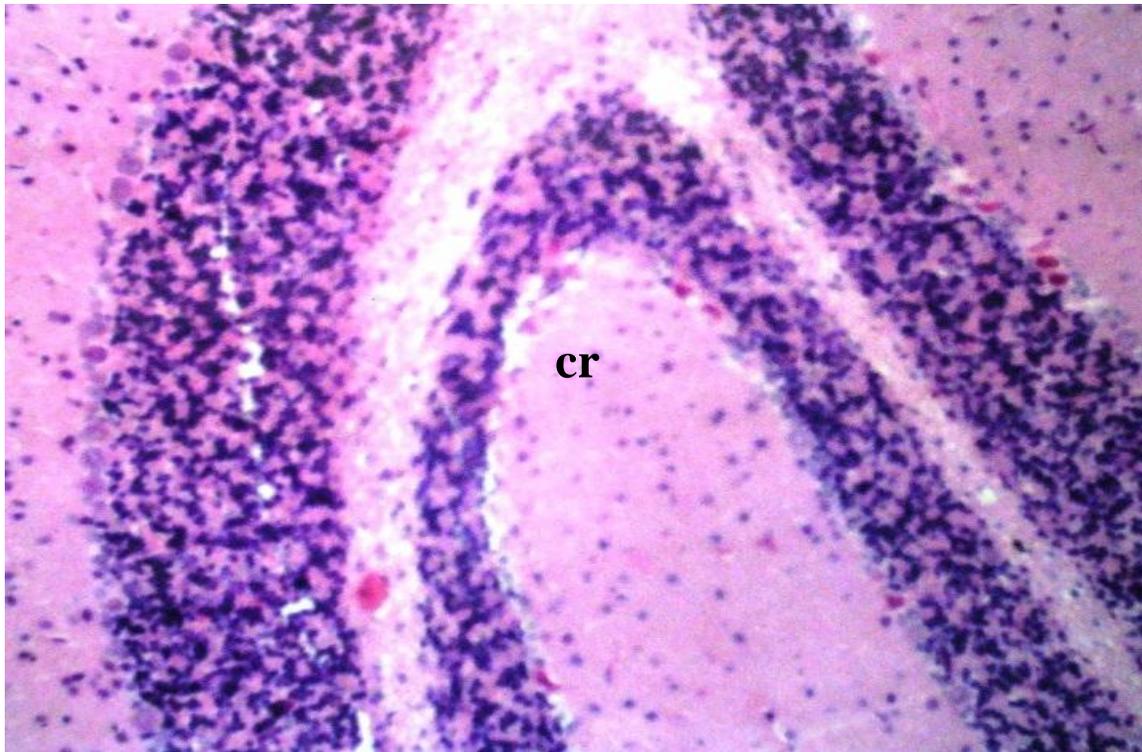


Figure 17. Brain section of control immature female rat showing the normal histological structure of the cerebellum (cr).

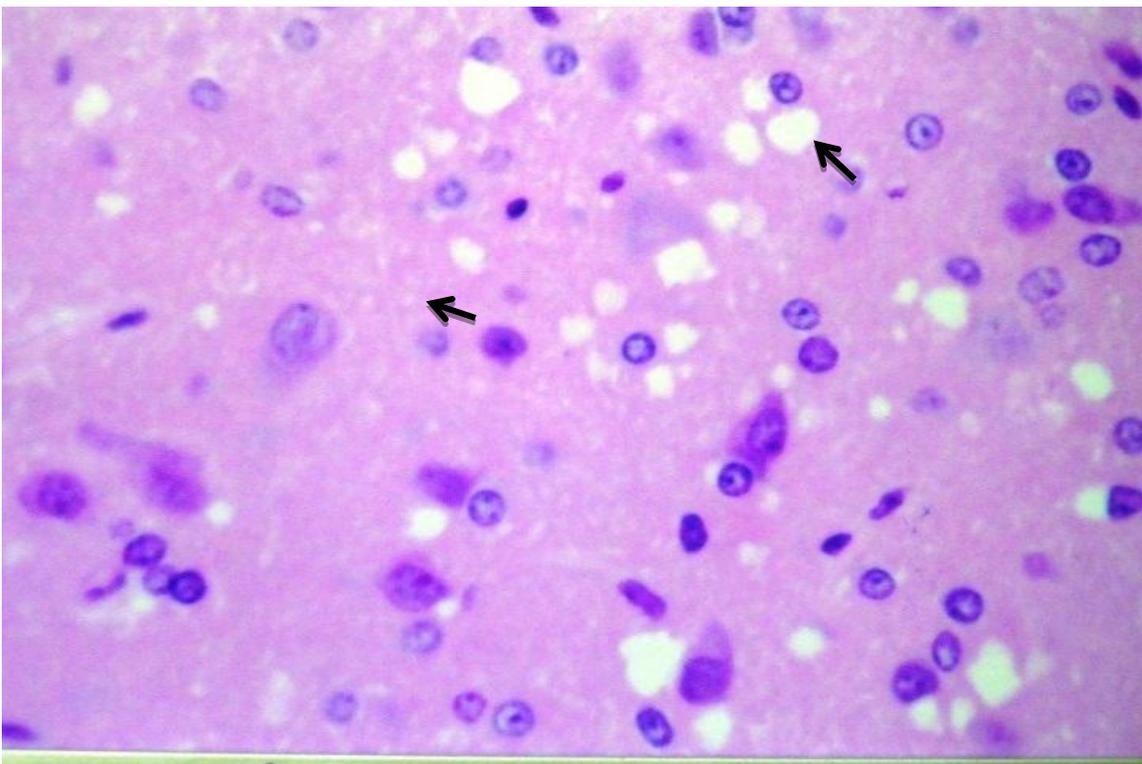


Figure 18. Brain section of immature female rat administered acrylamide for 28 days showing vacuolation in the cerebral matrix (arrow).

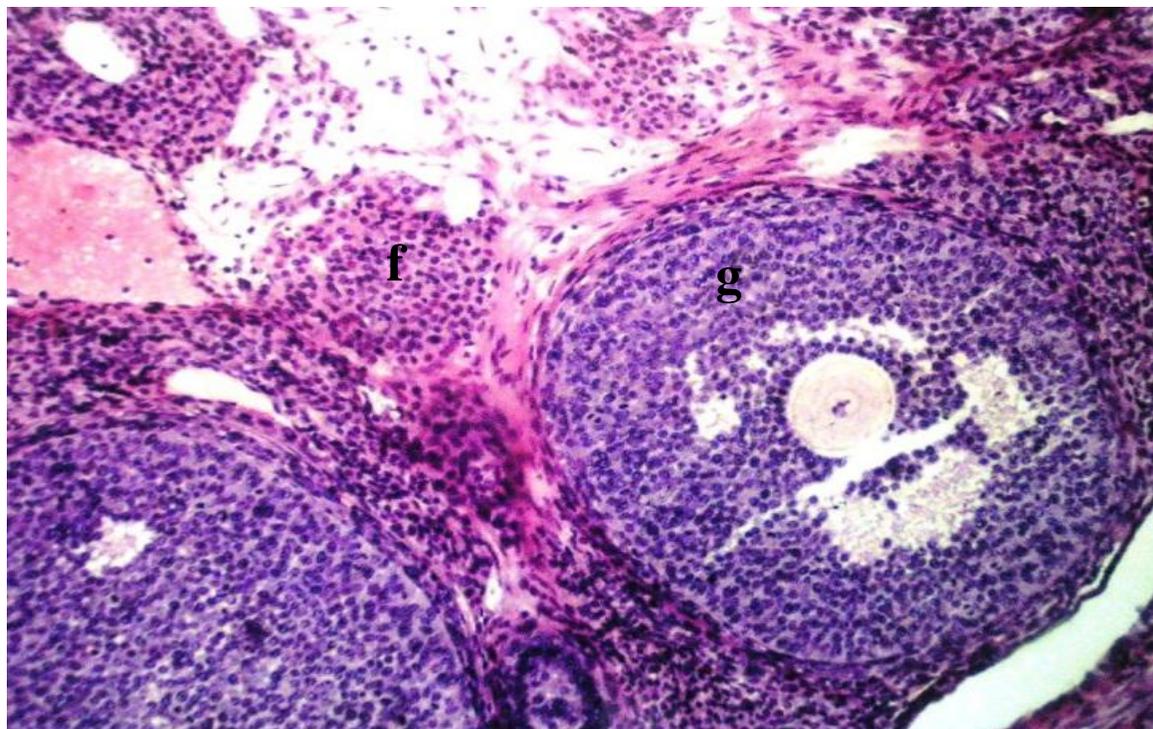


Figure 19. Ovary section of control immature rat showing the normal histological structure of the graafianfollicles (g) and interfollicular cells (f).

treated rats for ten days. The authors attributed hind limb paralysis to ACR neurotoxicity.

The principal effect of acrylamide monomer was neurotoxicity. It has been shown to produce a central-peripheral neuropathy in laboratory animals including rats and monkeys, as well as in humans (LoPachin, 2004). El-Yamany and Bayomy (2007) concluded that all the symptoms accompanying ACR intoxication in rats such as abnormal body posture, muscle weakness and legs play may be related to the degeneration of brain monoaminergic system, these findings supported by the present investigation which indicated that administration of ACR induced alteration in whole brain monoamines concentration.

Hind limb dysfunction and abnormal gait recorded in the present study are supported by the histopathological findings following ACR administration. These changes varying from focal gliosis in the cerebral cortex and cerebrum, focal hemorrhage in the meninges and vacuolization was detected in cerebral cortex, cerebrum, cerebellum and medulla oblongata. It can be also shown that the effect of ACR on the brain tissue was dose and time dependent.

Body weight is frequently the most sensitive indicator of adverse effects of xenobiotics. So, it is considered as a destruction hemoglobin. As reported by Bergmark et al. (1993) and Barber et al. (2001), acrylamide is electrophilic and covalently binds to the cysteine residues

and forms adducts with sulfhydryl groups on hemoglobin determinant parameter of toxicity testing (Ali et al., 1992). In conjunction with the reports of Sharma and Jain (2008); Park et al. (2010) and Raju et al. (2011), the results of the present study clearly demonstrated that, oral administration of ACR to immature male and female rats induced significant reduction of body weight. These results are in agreement with the results reported by Wang et al. (2010), who suggested that acrylamide exerts detrimental effect on growth and development of immature male rats. Another explanation of body weight retardation may be resulted from total protein deficiency recorded in the present investigation. This is consistent with Abdul-Hamid et al. (2007) who suggested that, the reduction of body weight resulted from growth and protein deficiencies due to malnutrition during the development. It also, may have resulted from excessive break down of tissue proteins (Chatterjea and Shinde. 2002) or decreased in both plasma and tissue proteins (Yousef and El-Demerdash, 2006).

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including acrylamide on the blood constituents of an animal. Such analysis is relevant to risk evaluation as the changes in the hematological system have higher predictive value for human toxicity, when the data are translated from animal studies (Olson et al., 2000). In conjunction with reports of Tarskikh

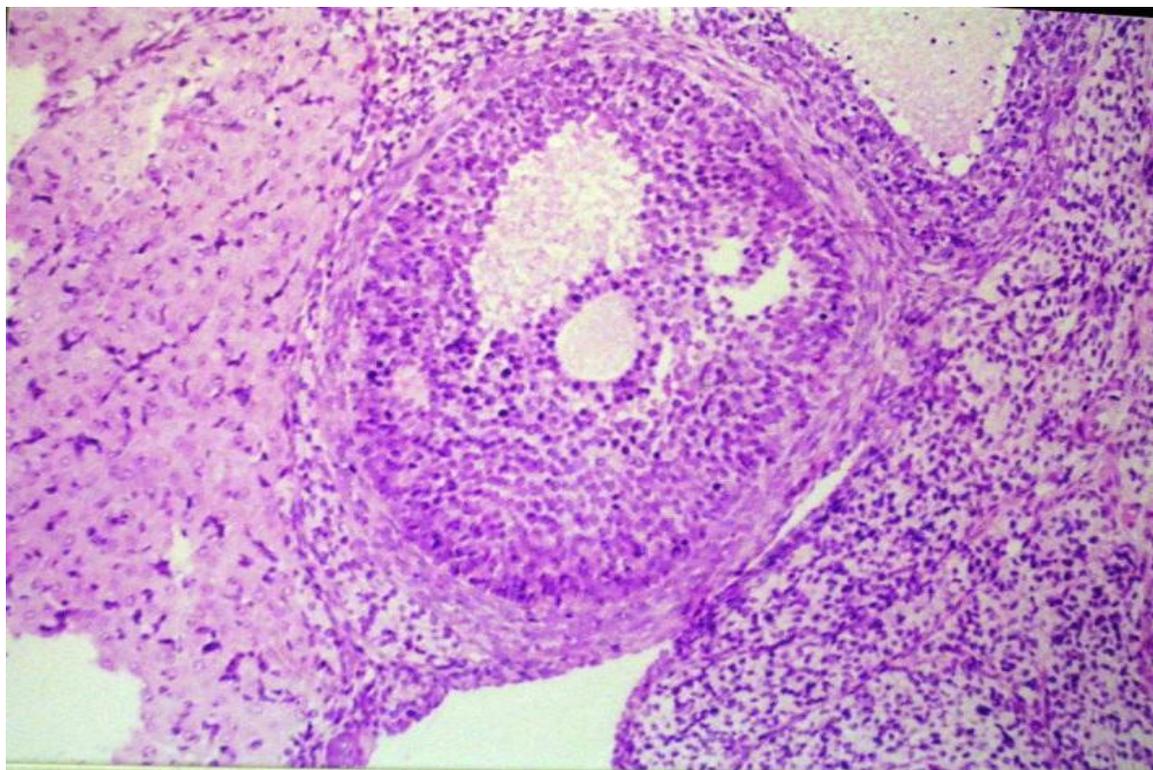


Figure 20. Ovary section of immature rat treated with acrylamide for 28 days showing mature follicle (g) and corpus luteum (cl).

(2006), Sharma and Jain (2008) and Arihan et al. (2011), the results of the present study revealed that, administration of ACR (15 mg/kg b.wt.) in immature male and female rats following 28 days and as compared to their control, caused decrease in erythrocytes count (RBCs). Acrylamide is not only neurotoxic and carcinogenic, but it also damages erythrocyte membrane and generates micronucleated erythrocytes as well as alters blood viscosity parameters (Arihan et al., 2011).

Tarskikh (2006) observed changes in physiochemical characteristics of biological membranes, decrease in erythrocyte acid resistance and activation of lipid peroxidation at the early stage after ACR administration. Moreover, the author noted that these changes are accompanied by a decrease in erythrocytes count. It may be suggested that intoxication with ACR is followed by liver disorders and condensation of erythrocyte membranes, which probably result from cholesterol accumulation due to liver dysfunction (Sniedze and Vasarinya, 1988).

Since, the hemoglobin (Hb) is linked to the total population of red blood cells, the present study revealed that Hb decreased significantly after oral administration of ACR at the tested dose (15 mg/kg b.wt.) following 28 days in both immature male and female rats. Low levels of Hb might be either due to the retarded synthesis or destruction hemoglobin. As reported by Bergmark et al.

(1993) and Barber et al. (2001), acrylamide is electrophilic and covalently binds to the cysteine residues and forms adducts with sulfhydryl groups on hemoglobin resulting in the loss of heme part of hemoglobin molecules thereby reducing the amount of Hb in the blood, which in turn may also be responsible for the anaemic conditions as evident by the low levels of RBCs observed in the present investigation.

As reported by Gargas et al. (2009), acrylamide and its epoxide glycidamide form adducts with (NH₂-terminal) NH₂ group of the valine residue of Hb. Acrylamide form adduct with hemoglobin so it leads to disturbance in serum iron concentrations (konings et al., 2003). The previous changes were confirmed by Sharma and Jain. (2008), as they concluded that ACR completely disturbed the equilibrium of hematological and thyroid hormonal status.

The hematocrit (HCT) depends on the erythrocyte mass, mean corpuscular volume and plasmatic volume. Generally, when the erythrocytes have a normal size, the modifications of HCT follow the Red cell distribution width changes (Perkins, 2004). The present study indicated that HCT values were decreased significantly subsequent to ACR administration (15 mg/kg b.wt.) in immature male and female rats. Similarly, some previous investigators indicated that, HCT value of rats was found to be decreased due to treatment with ACR (Sharma and Jain,

2008).

The present study also showed significant reduction in the total white blood cell count (WBC) following ACR treatment in both male and female rats after 28 days. This decrease in total WBC count suggests that ACR may be immunosuppressive. This reduction could be due to their diminished production, redistribution from peripheral blood into the tissues or rapid destruction of WBC (Debaun, 2005). The reduced neutrophils will adversely affect the phagocytosis activity in the animals. Similarly, decreased levels of basophils observed in the present study may suggest adverse effect on the immune system.

Biochemical parameters are sensitive index to changes due to xenobiotics and can constitute important diagnostic tools in toxicological studies. Blood glucose is a sensitive index to type and time of toxicity (Hanna et al., 1997). The present study showed that, serum glucose concentration of immature male and female rats increased significantly and exhibit strongly positive correlation with the experimental time after ACR treatment, these results are confirmed by the reports of Lin et al. (2009); they attributed the increase in serum glucose level to a decrease in blood insulin and insulin resistance status, which is associated with ACR intoxication.

The site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis (Bandyopadhyay et al., 1999). In accord with the studies of Yousef and E-Demerdash (2006); Sharma and Jain (2008) and El-Bohi et al. (2011), the present study showed that ACR decreased the serum total protein content. It was reported that hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases (Koneri et al., 2008), hence decline in total protein can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases as manifested by the sever histopathological alterations of the liver tissue following ACR treatment. ACR molecules has two reactive sites, via, the conjugated double bond and the amide group which can conjugate with the -SH group of a sulfur containing amino acids and α -NH₂ group of a free amino acid. The above scenario can explain the unavailability of few amino acids for protein synthesis. Further being an electrophilic compound ACR can bind with proteins which can make them undetectable.

Lipids have structural and functional important roles in different body organs and cells. The lipid is an important part of healthy body because it is used to form cell membranes, several hormones and is necessary for other cellular function. The observed increase in total cholesterol level may result from the decline in HDL-cholesterol level or from increased liver fatty acid synthesis (Gans, 1973). Therefore, high serum cholesterol level can be due to hepatic dysfunction. In

conjunction with reports of Khalil and Abd El Aziem (2005) and Allam et al. (2010), the present study revealed that serum total lipids, total cholesterol and LDL-cholesterol increased significantly following ACR administration in immature male and female albino rats. These results indicated that changes in plasma lipoproteins can serve as sensitive and simple markers for rats liver disorders caused by acrylamide. Also, the changes in the cells of the treated groups were observed in the histopathological investigations.

The marked decreased serum triglycerides concentration may be explained on the basis of decreased formation of plasma lipoproteins at an early stage after the liver injury (Seakins and Robinson, 1963). It is possible that an adequate synthesis of the lipoproteins by the liver is a prerequisite to the believed that the synthesis of lipoproteins occurs at the endoplasmic reticulum from triglycerides synthesized elsewhere (Heimberg et al., 1962).

The renal profile was investigated in this study to determine if ACR induces renal damage. In conjunction with the reports of Khalil and Abd El Aziem (2005), the present study showed that, administration of ACR induced some alterations in the serum creatinine, urea and uric acid compared to untreated groups. This agrees with the results of Shelly (1996) who observed a transient impairment in renal function following acute ingestion of ACR. These findings are supported by the histopathological investigation which showed severe congestion in the cortical blood vessels associated with swelling and degeneration in the epithelial cells lining the tubules. Continuous administration of ACR induced focal haemorrhages in between the tubules at the corticomedullary junction associated with focal fibrosis in between other tubules.

Serum ASAT and ALAT are the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar, 2002). During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage (Pari and Murugan, 2004). In conjunction with previous reports (Khalil and Abd El Aziem, 2005; Sharma and Jain, 2008; Allam et al., 2010; El-Bohi et al., 2011) the results of the present investigation showed increment in serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities following ACR treatment in immature male and female rats as compared to their corresponding controls. These results confirmed by the hypothesis recorded by Chinoy and Memon (2001), who attributed the increase in the serum ASAT and ALAT activities to the bipolar nature of ACR, where the CH₂=CH part may undergo hydrophobic interactions while the CONH₂ part can form hydrogen bonds with the cell compounds. This property may enhance its ability to alter the cell membrane structure and make the parenchymal cell

membrane of liver more permeable. Therapy causing the active retention of enzymes and making them appear first in the extracellular space and then in the blood. The previous changes were confirmed by the histopathological findings. The histopathological alteration following ACR treatment induced focal areas of degeneration, vacuolation, fatty change in the hepatic parenchyma and associated diffuse kupffer cells proliferation as well as inflammatory cells infiltration in between the degenerated hepatocytes. The present study confirmed the finding of Veenapani et al. (2010) who showed histopathological changes in liver following ACR administration. These changes vary from central vein congestion, degenerative changes, sinusoidal haemorrhages, hyperplastic hepatocytes to binucleated hepatocytes on microscopic analysis.

On the other hand, alkaline phosphatase (ALP) activity was declined in the present study following ACR treatment in immature male and female rats. These results are in agreement with Yousef and EL-Demerdash (2006) and Allam et al. (2010). The inhibitory effects of ACR on ALP activity might result from abnormalities in its gene expression where acrylamide form adduct with the hepatic cell's DNA as reported by Dybing and Sanner (2003). Also, ACR leads to DNA strand breaks and dominant lethal mutations (Sega et al., 1989 and Tyl et al., 2000).

The present result indicated that, treatment with acrylamide at (15 mg/kg b.wt.) caused inhibition in the activity of acetylcholinesterase (AChE) in the serum of treated rats. This result is in accordance with the finding of Yousef and EL-Demerdash (2006). AChE is the presynaptic (cholinergic) and postsynaptic (cholinceptive) components of these pathways where it terminates the synaptic action of acetylcholine through catalytic hydrolysis. LoPachin et al. (2004) suggested that acrylamide-induced synaptic dysfunction which involved the adduction of presynaptic protein thiol groups and subsequent reduction in neurotransmitters release. Barber and LoPachin (2004) reported that the neurological defects associated with acrylamide intoxication are mediated by impaired neurotransmission at central and peripheral synapses.

Evidences suggested that the cumulative neurotoxicity produced by ACR exposure is linked to nerve terminal damage in the central nervous system and peripheral nervous system (Lehning et al., 2003; LoPachin et al., 2003). ACR neurotoxicity was found to be mediated by injury to nerve terminals and cerebellar purkinje cells (LoPachin, 2004). Consequently, the present study has been carried out in an attempt to investigate the effects of acrylamide on neurotransmitters (catecholamines: norepinephrine, dopamine and serotonin – free amino acids: aspartate, glutamate, glycine (GABA), taurine and alanine) contents in whole brain in order to clarify the brain function.

The observed data concerning norepinephrine level in

the present study showed a significant increase in whole brain of immature male and female rats after 28 days following ACR administration as compared to control groups. This result is in agreement with LoPachin (2004), LoPachin et al. (2004) and El-Yamany and Bayomy (2007) study. LoPachin (2004) and LoPachin et al. (2004) reported that both low and higher doses of ACR inhibited the release of neurotransmitters and attributed this inhibition to the formation of ACR adducts with many proteins involving the neurotransmitter release. On the other hand, the reduction in norepinephrine level post ACR treatment may be attributed either to the capability of ACR in interacting with amino acids, tryptophan and tyrosine, the precursors of biogenic amines synthesis (Dixit et al., 1980) or to the increase of monoamine oxidase activity, the rate limiting enzyme of monoamines catabolism (Dixit et al., 1981).

In accord with the studies of El-Yamany and Bayomy (2007) and Tareke et al. (2009), the present study demonstrated that oral administration of ACR induced high significant reduction in whole brain dopamine concentration following ACR treatment (15 mg/kg b.wt) in immature male and female rats. Dixit et al. (1981) and Husain et al. (1987) reported that subchronic dose of ACR reduced the dopamine level in whole brain of developing rats and in the hypothalamus, pons and medulla oblongata of mature rats; they attributed this reduction to the increase in monoamine oxidase activity which is responsible for the increase of dopamine catabolism.

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter and biochemically derived from tryptophan. Serotonin is primarily found in the gastrointestinal (GI) tract (it synthesized in the intestinal chromaffin cells), platelets, and in the central nervous system (CNS) of animals including humans. It is a well-known contributor to feelings of well-being; it is also known to contribute to happiness (Young, 2007). In conjunction with the report of Mannaa et al. (2006), data observation in the present study indicated that there was highly significant decrease in whole brain serotonin level in the immature male and female rats following ACR (15 mg/kg b.wt) treatment. The decrease in serotonin in ACR-treated rat supported the other findings reported by other investigators that ACR induces severe nerve damage in the central nerve system (Ali et al., 1983).

Mannaa et al. (2006) indicated that ACR treatment resulted in a significant decrease in serum corticosterone suggesting that the adrenal cortex undergoes severe effects and/or ACR induces disturbances in the hypothalamic-pituitary-adrenal relationships. El-Yamany and Bayomy (2007) concluded that all the symptoms accompanying ACR intoxication in rats such as abnormal body posture, muscle weakness and leg splay may be related to the degeneration of brain monoaminergic system. The biochemical basis for the neuropathy may also involve modification of amino acids and proteins

present in neurons and suppression of amino acid incorporation into proteins of the nervous system (Hashimoto and Ando, 1973, 1975). The present findings revealed that acrylamide (15 mg/kg b.wt) neurotoxicity is mediated by increased neurotransmitters concentration in the whole brain including aspartate and glutamate of immature male and female rats.

LoPachin et al. (2003) and LoPachin (2004) concluded that ACR administration induced defective neurotransmission at central and peripheral synapses of ACR-intoxicated animals. This defect might be mediated by changes in presynaptic transmitter uptake, synthesis, vesicle storage or release (Goldstein, 1985). The results obtained by LoPachin (2004) showed that K^+ -evoked, Ca^{2+} -dependent glutamate release was significantly decreased in synaptosomes isolated from ACR-intoxicated rats, whereas the kinetic parameters of high affinity, Na^+ -dependent glutamate uptake were not consistently affected.

The present study indicated that taurine concentration of immature male rats increased significantly subsequent to ACR administration for 28 days at low dose (15 mg/kg b.wt). LoPachin et al. (2002, 2003) proposed that the nerve terminal is the primary site of acrylamide action leading to inhibition of neurotransmission and the resulting neurotoxicological consequences. Their proposed mechanism of the inhibition of neurotransmission at central and peripheral synapses is based on adduct formation between acrylamide and cysteine-rich terminal proteins that mediate fusion of membranes during exocytosis.

In conclusion, acrylamide is one of the major environmental public health problems as a result of increased accumulation in processed cooking food materials. The present study showed that, acrylamide induced hematological, biochemical, neurological and histopathological disorders in the immature male and female rats. So, we recommended that children must eat balanced diet, rich in fruits and vegetables and avoid fast or junk foods. We also advise that food should not be cooked excessively for a long time or at too high temperature.

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