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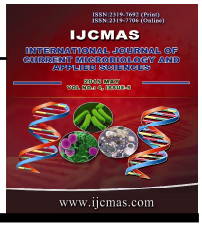

Original Research Article

Effect of Acrylamide on Liver and Kidneys in Albino Wistar Rats

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Keywords

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A B S T R A C T

Acrylamide (ACR) is a chemical with a very wide range of uses and it accounts for one of the major health concern because it has been detected in a widely consumed food item, fried breads (or any carbohydrate-rich food items cooked at high temperature) accordingly the general population is highly exposed to ACR. Four groups of adult female rats were used in this study to investigate the effects of different doses of ACR administration on the liver and kidneys. Group1: Control was given distilled water; group 2: low dose given 2mg /kg/day; group 3: mild dose given 10mg/kg/day and group 4: high dose given 30mg/kg/day ACR, the doses were given orally for eight weeks. Results showed no significant differences in serum urea and creatinine level of the low dose group, while mild and higher dose groups showed significant decreases compared with control. No significant differences recorded in T.S. bilirubin levels in all treated groups. S. uric acid levels significantly increased in the low dose group, but its level significantly decreased in both mild and high dose groups compared to the control. Levels of each of S. total protein, S. albumin, S. globulin, S.G.P.T., S.G.O.T. and S.A.L.P was significantly decreased in all treated groups compared with control. Histological examinations of the liver and kidneys of the all groups treated with ACR revealed degeneration of the glomerular tuft, the epithelial lining of the kidney's brush border membrane and liver hepatocyte. Both liver and kidneys showed congestion in the blood vessels. For the first time, three different doses of acrylamide have been investigated, and tested for both physiological and histopatholgical changes. It has been concluded that body can tolerate and detoxificat low ACR doses. While mild and high doses lead to degenerative changes and physiopathological disturbance, indicate the local action of ACR on both the liver and kidney cells and remodeling as they are the main sites of detoxification and excretion of toxic materials of administering substances. A significant decrease in body weight in Wistar rat treated with various concentrations of ACR in comparison to control groups. This is probably the result of breakdown of tissue and blood cells in the liver and kidney by ACR in Wistar rats.

Introduction

Acrylamide (ACR) is a reactive, small organic molecule with very high water solubility. These properties facilitate its rapid absorption and distribution through the body (Mannaa *et al.*, 2006). ACR represents an industrial chemical used in the manufacturing of polyacrylamides that are common in personal care and grooming products (e.g. lotions, cosmetics, deodorants), soil conditioning, wastewater treatment, and paper and textile industries (Exon, 2006; Friedman, 2003). In addition to such industrial and laboratory uses, high levels of ACR were detected in tobacco smoke (Pruser and Flynn, 2011).

ACR has become one of the major public health concerns since it was detected in widely consumed food items; for example, fried bread (breakfast cereals), potato chips, and any carbohydrate-rich food items cooked at high temperatures (higher than 200°C)(Guyton and John, 2006; Kaneko *et al.*, 1997).

The toxicity of ACR is attributed to the fact that it biotransforms to a more potent and highly reactive molecule that initiates cellular toxicity. Therefore, the most important pathogenic pathway is the oxidative biotransformation of ACR by cytochrome P450 2E1 (CYP2E1) (Hammad *et al.*, 2013). The resulting metabolite is an epoxide derivative, glycidamide, which is more reactive towards DNA and proteins than the parent compound, ACR (El-Mottaleb and Rashed, 2008; Elaimy, 2006). The majority of ACR is conjugated with glutathione while a lesser amount is activated via glycidamide (Kaneko *et al.*, 1997).

Once absorbed, ACR may be conjugated by glutathione-S-transferase (GST) to N-acetyl-S-(3-amino-3-oxopropyl) cysteine or it reacts with cytochrome P450 (CYP450) to produce glycidamide. Several metabolic studies have been conducted that focused on the interaction of ACR with CYP450 and GST in rats and mice (Alturfan *et al.*, 2011; Rawi *et al.*, 2012; Khalil, 2005). The results of these studies indicated that liver, kidney, brain and erythrocyte GST have significant binding capacity with ACR, with liver GST is three times more efficient in conjugating ACR compared to brain GST in rats (Alturfan *et al.*, 2011). Additional studies in rats indicate that ACR may inhibit GST, resulting in increased metabolism of glycidamide by the CYP450 pathway.

Since ACR is formed during the cooking or frying of many commonly consumed foods at high temperatures, the general population is highly exposed to ACR in their diets. This study aimed to investigate the effects of ACR administration on the liver and kidneys of rats that were given different doses orally for eight weeks compared to control rats.

Materials and Method

Animals & Housing

The experiment was conducted on forty female Albino Wistar rats, weighing 280–300 gm. Animals were acclimated for about one week and housed in plastic cages. During the experimental period, they were housed under standard laboratory conditions, 12:12 light/dark photoperiod at 23 ± 2 ºC. The animals had ad libitum access to water and diet through the study time.

Experimental design

This experiment was designed to study the effect of ACR administration on the liver and kidneys. The animals were assigned to

one of the four groups, each with ten rats as follows:

1. Group 1: control group, rats were given distilled water.
2. Group 2: low dose ACR, rats were given 2 mg/kg of body weight/day.
3. Group 3: mild dose ACR, rats were given 10 mg/kg of body weight/day.
4. Group 4: higher dose ACR, rats was given 30 mg/kg of body weight/day. The applied dose was selected according to Tyl and Friedman (Khalil, 2005).

ACR and distilled water were given orally by gavage for eight weeks. After eight weeks the animals were euthanized:

A. The blood was collected; serum from non-heparinized blood was used for various kidney function tests (urea, creatinine, T.S. bilirubin, and uric acid) and liver function tests (S. total protein, S. albumin, S. globulin, S.G.P.T., S.G.O.T., and S. alkaline phosphatase)

B. The liver and kidneys were excised and fixed in neutral buffered formalin 10%; the organs were routinely processed and sectioned at 4-5 mm thickness. The obtained tissue sections were mounted on glass slides, deparaffinized and stained with Hematoxylin and Eosin stain. The sections were then examined and observed under a light microscope at magnifications X100 and X400.

Statistical analysis

Biochemical parameters measurement: creatinine, urea, bilirubin, GPT, GOP, and ALP were determined by an Auto-Analyzer (LISA 200, France), using standard kits for each test. Statistical analysis of the data was performed by using SPSS (Version 18), using independent paired t-test.

Results and Discussion

The results, as recorded in the table 1, showed that there were no significant differences in urea levels between the control and the mild dose ACR groups, while there were significant decreases in both mild and high dose groups compared to the control group.

Similarly, there were no significant differences in S. creatinine level between the low dose ACR and the control groups, while both mild and high dose groups recorded a significant decrease compared to the control group.

On the other hand, T.S. bilirubin levels in all treated groups showed no significant differences compared to the control group. Unlike T.S. bilirubin, S. uric acid levels significantly increased in the lower dose ACR group compared to the control group but its level significantly decreased in both mild and high dose groups compared to the control.

Furthermore, S. total protein levels showed significant decreases in all treated groups compared to the control group. Likewise, S. albumin levels decreased significantly in all treated groups compared to the control group.

Similarly, all groups treated with ACR recorded significant decreases in S. globulin levels compared to the control group.

Likewise, S.G.P.T. levels significantly decreased in the low dose ACR group compared to the control group; significant decreases were also reported in both mild and high dose ACR groups compared to the control group.

Results showed the control group recorded a significantly higher level of S.G.O.T.

compared to the low, mild, and high dose ACR groups. In addition, S.A.L.P. levels significantly decreased in the low, mild and high dose ACR groups compared to the control group.

Histological results

The kidneys of the control rats showed the normal histological structure of the renal corpuscles and renal tubules. The renal corpuscle consisted of a tuft of blood capillaries surrounded by the Bowman's capsule. The latter had a parietal layer lined by squamous cells and a visceral layer lined by podocytes. The renal tubules included proximal convoluted tubules lined by large pyramidal cells with a brush border, distal convoluted tubules lined by cuboidal cells, loop of Henle, and collecting tubules (Figure 1A).

The kidneys of the groups treated with ACR showed degeneration of the glomerular tuft with infiltration of lymphocytes. The renal tubules became vacuolated and lost their brush borders; degenerative changes can be observed in their epithelial lining followed by rupture of the cells, necrosis, and finally congestion of the interstitial blood vessels (Figures 1B-D).

Control group sections of the liver revealed normal histological features with hepatic lobules. Each consisted of cords of regularly arranged hepatocytes enclosing the sinusoidal network and the central vein located in the center of the lobule. The hepatocytes were polygonal in shape and had a clear round to slightly oval nuclei with one or two nucleoli. The blood sinusoids were lined with non-parenchymal cells including Kupffer cells and endothelial cells. The portal area contained branches from the hepatic artery, portal vein, and bile duct (Figure 2A).

The liver of rats in the groups treated with three different doses of ACR showed degenerative changes of hepatocytes as well as congestion of the blood vessels (portal veins in the portal area) and infiltration of few mononuclear inflammatory cells, especially in the portal areas (Figures 2B-E). The liver of rats in the high dose treated group also showed a moderate infiltration of mononuclear inflammatory cells, congested blood vessels, and most of the hepatocytes were suffering from necrosis.

The degenerative changes observed in this study indicate the local action of ACR on both the liver and kidneys as they are the main sites of detoxification and excretion of toxic materials of administered substances.

The results showed that there were no significant changes in serum urea levels between the low dose ACR group and the control group; this may indicate that the kidney can perform to some extent even with a low dose of ACR. Furthermore, *chronic renal failure* results from progressive and irreversible loss of large numbers of functioning nephrons, but their clinical symptoms often do not occur until the number of functional nephrons falls to at least 70-75% below normal. In fact, relatively normal blood concentrations of most electrolytes and normal body fluid volumes can still be maintained until the number of functioning nephrons drops below 20–25% of normal (Guyton and John, 2006).

The results from the histopathological examination revealed necrosis of the epithelial lining of the renal tubules. This probably causes damage to less than 25% of the functioning nephron, which is why serum urea levels did not increase in low dose or even in mild and high dose ACR groups compared to the control group.

At mild and high ACR doses, there were significant decreases in serum urea levels since urea is biosynthesized in the liver from ammonia which derived from tissue or dietary proteins (Kaneko *et al.,* 1997). The decreased urea levels may be a result of impaired urea synthesis due to hepatic insufficiency because of liver damage (Agency USEB, 2011). The results agree with those recorded by Hammad *et al.* (2013), who used different doses of ACR (10, 30, 60, 90 mg/kg) for 6 weeks, and El-Mottaleb and Rashed (2008), who treated the specimens with 1/20 and 1/10 of LD50 of ACR for 28 days. El-Elaimy (2006) also mentioned that ACR, in 0.5 mg/kg dose of body weight, produced a non-significant decrease in creatinine and a significant decrease of urea values in rats. The results from present study disagrees with Alturfan *et al.* (2011); they reported that the BUN and creatinine levels significantly increased in the ACR treated group (40 mg/kg/day intraperitoneally) compared to those of the control group. This is probably because they used a higher dosage of ACR with a different route of administration that could have increased the absorption of the toxin and in turn increased the damage.

Creatinine levels showed a non-significant difference between the low dose ACR group and the control group, and a significant decrease in both mild and high dose ACR groups compared to the control group. Creatinine formation begins with the transamidination from arginine to glycine to form glycocyamine or guanidoacetic acid (GAA). This reaction occurs primarily in the kidneys, in the mucosa of the small intestine, and the pancreas. The GAA is transported to the liver where it is methylated by S-adenosyl methionine (SAM) to form creatine. Creatine enters circulation and 90% is taken up and stored by muscle tissue. In a reaction catalyzed by creatine phosphokinase (CPK), most of this muscle creatine is phosphorylated to creatine phosphate. Each day about 2% of these stores are converted, nonenzymatically and irreversibly, to creatinine. The decreased creatinine levels in the mild and high dose ACR groups could be due to an acceleration of these reactions in the liver and kidneys as part of ACR detoxification in these two organs; since creatinine is the product of muscle creatine catabolism and essentially reflects lean body mass, the phenomenon of muscle wasting and muscle dystrophy was quite obvious in rats given a mild or high dose of ACR due to the increased body demand to uptake much more creatine form muscle.

The results of the present work at 2 mg/kg are agree with the findings presented by Rawi *et al.* (2012); they reported that there were no significant changes between immature female rats treated with 15 mg/kg of ACR for 28 days and the control group regarding serum urea and creatinine levels Conversely, their work disagrees with this study on dose of 10 and 30 mg/kg with respect to the serum urea, uric acid, and creatinine levels; the reason for such disagreement may be because they used rats that were very young in age (21 days old) and had a shorter administration period (28 days).

The results showed an increase in uric acid levels in the low dose ACR group and, conversely, a significant decrease in both mild and high dose groups. This may be because the kidneys are the major site for removal of uric acid and accounts for two-thirds to three-fourths of its daily loss. ACR may impair the secretory mechanism and raise serum uric acid levels in low doses. In higher doses, ACR may inhibit urate absorption producing a urate diuresis and

reduction in serum uric acid levels. A low serum urate concentration may result from decreased production or increased excretion and since xanthine oxidase, the enzyme responsible for conversion of oxypurines to uric acid, is found in abundance in the liver and the mucosa of the small intestines, the enzymes rate of formation may decrease due to necrosis in regions of liver. The results agree with those of Khalil (2005) as they reported that the values of uric acid significantly decreased in groups that fed on a basal diet supplemented with potato crisps and toasted bread as source of ACR.

In this study there were significant decreases of the total protein, albumin, and globulin levels. The hypoproteinaemia in rats given different concentrations of ACR might have resulted from hepatocellular dysfunction. Evidence of liver damage was characterized by the development of cytoplasmic fatty vacuolation and necrosis of the centrilobular hepatocytes with lymphocytic infiltration. Asha *et al.* (2008) reported a steady decrease in hepatic protein levels with higher doses of ACR and attributed that to retarded protein synthesis, change in protein metabolism, or to the leakage of protein reserves from hepatocytes. An ACR molecule has two reactive sites, the conjugated double bond and the amide group which can conjugate with a thiol group of sulfur containing amino acid and α-NH2 group of a free amino acid (Friedman, 2003). This can explain the unavailability of few amino acids for protein synthesis. Furthermore, being an electrophilic compound, ACR can bind with proteins and make them undetectable.

The site specific oxidative damage of some susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis (Babu *et al.,* 2011). According to the studies of (El-Bohi *et al.,* 2011; Sharma and Jain, 2008; Yousef and El-Demerdash, 2006) hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases (Koneri *et al.,* 2008). Therefore, a decline in total protein can be a useful index of the severity of cellular dysfunction in chronic liver diseases, manifested by the severe histopathological alterations of the liver tissue, following ACR treatment.

In this study, alkaline phosphatase (ALP) activity declined following ACR treatment in female rats, decreased activity of ALT and AST enzymes in the tested groups could be attributed to decreased endogenous production or increased catabolism. These results agree with the findings of (Yousef and El-Demerdash, 2006; Allam *et al.,* 2010). The inhibitory effects of ACR on ALP activity might result from abnormalities in its gene expression where ACR forms adducts with the hepatic cell's DNA as reported by Dybing and Sanner (2003). Also, ACR leads to DNA strand breaks and dominant lethal mutations (Ao *et al.,* 2008; Tyla *et al.,* 2000).

The kidneys of rats in all groups showed infiltration of few mononuclear inflammatory cells as well as degenerative changes of some cells lining the renal tubules, other cells showed necrosis. These findings may be due to the fact that kidneys are the way of excretion of ACR and its metabolites. These results were similar to those reported by Totani and Mogda (Mansour *et al.,* 2008; Totani *et al.,* 2007). However, Jabbar, 2011 noted degenerative changes in the renal convoluted tubular epithelium in rabbits (2011) and AL-Mosaibih, 2013 recorded the degeneration and necrosis of hepatic parenchyma in rats that received large doses of acrylamide (AL-Mosaibih, 2013). Additionally, ACR causes oxidative stress by inducing the generation

of reactive oxygen species (ROS), thereby reducing the antioxidant defense systems of the cells by depleting non-enzymatic antioxidant systems (vitamins and glutathione) and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition (Lee and Jacobs, 2005). Also, the World Health Organization (WHO) (2002) reported that in rats, biotransformation of ACR occurs through glutathione conjugation and through decarboxylation; at least four urinary metabolites have been found in rat urine.

The liver of rats in the low and mild dose ACR groups showed degenerative changes of the hepatocytes, as well as congestion of the blood vessels and infiltration of few mononuclear inflammatory cells. The liver of rats in the high dose ACR group showed moderate infiltration of mononuclear inflammatory cells, congested blood vessels, and most of the hepatocytes were suffering from necrosis. These results agree with the findings of authors (El-Mottaleb and Rashed, 2008; Mansour *et al.,* 2008). These findings stated that ACR generated ROS which enhanced lipid peroxidase production; cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroxides (Al-Serwia and Ghoneim, 2015). Lipid peroxyl radicals can subsequently propagate into malondialdehyde (MDA) that is also used to investigate the oxidative damage of proteins and lipoproteins which is a possible mechanism for liver injury (Ayala *et al.,* 2014). Also, Park *et al.* (2002) reported that a reduction of liver cellular glutathione (GSH) levels due to ACR treatment was observed.

From the present work we can conclude that in spite of some necrosis observed in the renal tubule lining of a low dose of ACR rats (2 mg/kg/day) kidneys performance were sill not affected, indicated through both urea and creatinine levels, while mild (10 mg/kg/day) and high doses (30 mg/kg/day) exert a potential effect enough to cause renal and hepatic insufficiency indicated through decreased urea and creatinine levels confirmed by the histological examinations. The decreased creatinine levels for both mild and high dose ACR were great enough to cause obvious muscle dystrophy due to the increased body demand to uptake much more creatine form muscle. The damaged liver and kidneys results in the increased uric acid levels for the low dose ACR and the decreased level in both mild and high doses ACR.

Table.1 Compare physiological parameters between control group and (mild and high dose groups)

Parameters	**Control Mean±SE**	**Low dose (2mg)Mean±SE**	**Mild dose (10mg) Mean±SE**	**High dose (30mg) Mean±SE**
S. urea	52 ±1.72	50.5 ±2.0	39 ±1.6***↓**	35.4 ±2.8***↓**
S.creatinine	0.72 ±0.019	0.75 ±0.022	0.43 ±0.033***↓**	0.53 ±0.021***↓**
T.S. bilirubin	0.14 ±0.020	0.15±0.022	0.16±0.020	0.16±0.021
S.uric acid	3.9±0.7	5.5±0.37***↑**	2.7±0.30***↓**	3.1±0.27***↓**
S.total protein	8.4±0.25	7.4±0.17***↓**	7.3±0.12***↓**	6.6±0.06***↓**

S.albumin	4.4±0.15	3.7±0.12*↓	3.7±0.06*↓	3.6±0.03*↓
S.globulin	4.2±0.12	3.6±0. 11*↓	3.5±0.1*↓	3.0±0.03*↓
S.G.P.T.	63 ±2.42	47 ±2.81*↓	37 ±3.80*↓	41 ±2.31*↓
S.G.O.T.	194 ±1.85	142 ±7.19*↓`	166 ±2.60*↓	148 ±2.83*↓
S.alkaline phosphatase	899.7 ±41.4	656.1 ±10.2*↓	542.5±20.6*↓	410.6±31.2*↓

* represent a significant difference *(p□0. 05)* compared with control

Figure.1A. Kidney of a control (normal) rat showing normal histology of a glomeruli and renal tubules. **B.**Kidney of a low dose treated rat; congestion of the interstitial connective tissue, blood vessel (black arrow) with congestion of the capillary tuft (glomerulus) (blue arrow). **C.** Kidney of a mild dose treated rat;necrosis of the epithelial cells of kidney tubules (red arrow), swelling or hydropic degeneration leading to rupture of the epithelial lining of tubules (black arrow). **D.** Kidney of a high dose treated rat; severe congestion of interstitial blood vessels (black arrow), necrosis of the epithelial cells of kidney tubules (red arrow) and infiltration of few mono-nuclear inflammatory cells (blue arrow), (hematoxylin and eosin stain, X400).

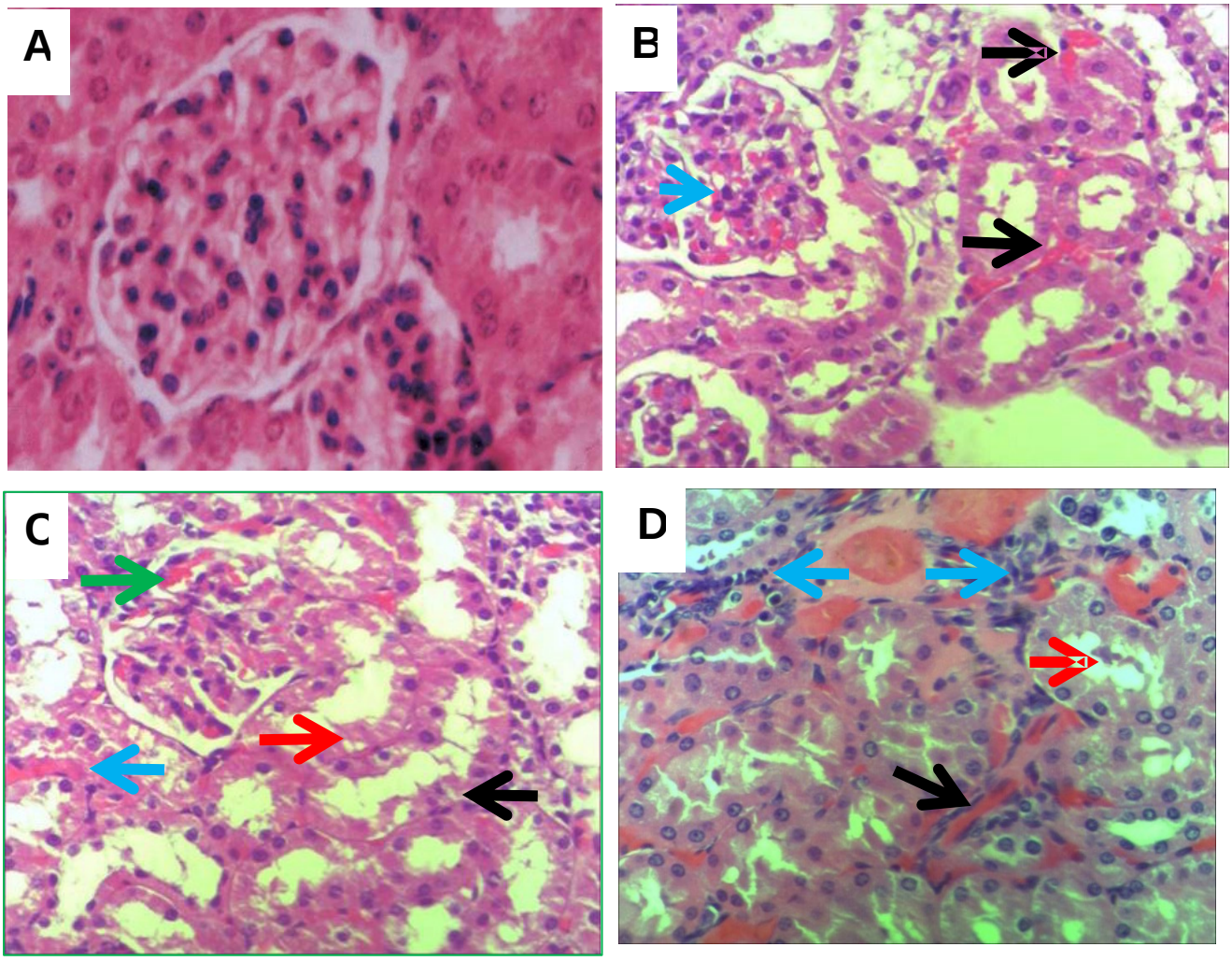
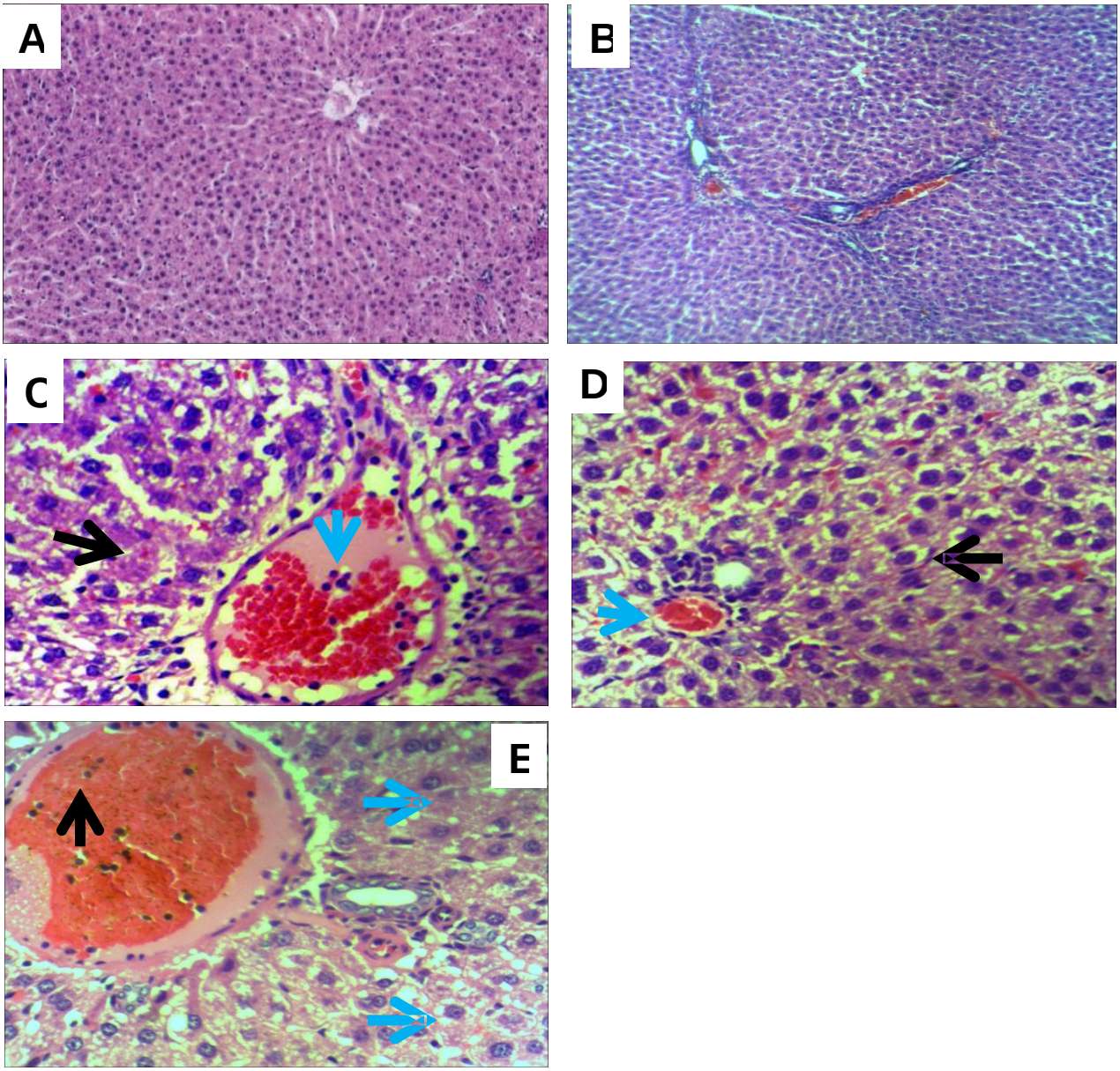


Figure.2A Liver of a control (normal) rat showing normal histology of a hepatic lobule. **B.** Liver of a low dose treated rat; congestion of the interlobular connective tissue blood, vessels and a branch of the hepatic artery within the portal area, (hematoxylin and eosin stain, X100). **C.** Liver of a mild dose treated rat; congestion of the interlobular connective tissue, blood vessels (blue arrow) with obvious swelling or degeneration of the hepatocytes (black arrow). **D.** Liver of a high dose treated rat; congestion of the liver sinusoids and a branch of the hepatic artery in the portal area (blue arrow) with swelling or degeneration of the hepatocytes leading to push the nuclei to periphery (black arrow). **E.** Liver of a high dose treated rat; congestion of the central vein (black arrow) and swelling or degeneration of the hepatocytes led to necrosis surrounding the central vein (blue arrow), (hematoxylin and eosin stain, X400)



Total serum protein, albumin, and globulin levels decreased in all groups treated with ACR compared to the control group, due to retarded protein synthesis or changes in protein metabolism which is accounted for another indicator for hepatocellular dysfunction. Though ACR may cause abnormalities in gene expression of hepatic cells, the results were showed decreased levels of ALP, GPT, and GOT in all groups treated with ACR compared to the control group. A significant decrease in body weight in rat treated with various concentrations of ACR in comparison to control groups. This is probably the result of breakdown of tissue and blood cells in the liver and kidney by ACR in Wistar rat.

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