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Protective Effect of Ascorbic Acid, Biopropolis and Royal Jelly against Aluminum Toxicity in Rats

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Abstract

The present study was conducted to investigate the effectiveness of ascorbic acid (AA), biopropolis and royal jelly in alleviating the toxicity of Aluminum chloride ($AlCl_3$) on body weight gain, feed efficiency and biochemical parameters in rats with hostological examination of sections from liver and kidney. Thirty female Wistar-Albino rats (160-170 g) were randomly divided into five equal groups of six rats each. As normal drinking water was given to the control group, while the four groups II, III, IV and V received 34 mg AlCl3/ L in drinking water daily. Group II received only $AlCl_3$. Group III, IV and V were administrated orally with ascorbic acid, biopropolis and royal jelly, respectively at a dose 50 mg/kg bw twice a week for 8 weeks. $AlCl_3$ decreased significantly body weight gain and feed efficiency. While treatment of ascorbic acid (AA), propolis and royal plus $AlCl_3$ normalized their to control value. The relative weight (percent of body weight) of liver and kidney increased when compared with control while the brain did not illustrate any change. The level of urea, and the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were increased, while total protein and albumin were decreased in serum of rats treated with $AlCl_3$. It can be concluded that ascorbic acid, propolis and royal jelly have beneficial influences and could be able to antagonize $AlCl_3$ toxicity.

Keywords: Aluminum chloride: toxicity: ascorbic acid: biopropolis: royal jelly: rats

1. Introduction

Aluminum (Al), the third most common element approximately 8% of total mineral components in the earth's crust found combination with oxygen, silicon, fluorine and other elements in the soil, rocks, clays and gems has a significant toxic potential for humans (Verstraeten *et al*., 2008). Al is widely distributed in the environment and extensively used in daily life, which causes its easy exposure to human beings (Kumar and Gill, 2009). It gets access to the human body via the gastrointestinal and the respiratory tracts. Al is a constituent of cooking utensils and medicines such as antacids, phosphate binders, buffered aspirins, vaccines, antiperspirants, allergen injection (Exley, 1998)., deodorants and toothpaste (Abbasali, et al., 2005). Food and food additives contain small but variable amounts of Al (Reinhold, 1980) and this has allowed its easy access into the body (Yokel, 2000).

Al absorption/ accumulation in humans can occur via the diet, drinking water, ingestion with fruit juices or citric acid causes a marked increase in both gastrointestinal absorption and urinary excretion of Al in healthy subjects (Venturini-Soriano and Berthon, 2001). The sources of Al are especially corn, yellow cheese, grain products (flour), salt, herbs, spices, tea leaves, many food products, vegetables, cereals, beverages cosmetics, cookware, cans and containers. Also, Al is added to drinking water for purification purposes (Turkez et al., 2010) Thus urban water supplies contain greater concentration of Al ions. Al accumulates in all tissues of the mammals, including kidney, liver, heart, blood, bone and brain (Al-Kahtani, 2010). Kidney plays a major role in preventing accumulation of Al by excreting it out through urine (Stoehr et al., 2006). Al is one of the most studied neurotoxicant affecting nervous system, including various regions of brain (Nehru and Bhalla, 2006). Some experts believe that Al plays a role in the formation of Alzheimer like neurofibrillary tangles (Sharma et al., 2009). Accumulation of Al in liver produces hepatic injury at higher concentrations (Shati and Alamri, 2010). Because of the cumulative nature of Al in the organism after dietary exposure, the European Food & Safety Authority (EFSA) (2008) established a tolerable weekly intake (TWI) for Al instead of a tolerable daily intake. The EFSA concluded recently that the estimated dietary exposure to Al in the general population in several European countries is likely to exceed the TWI in a significant part of the European population. Al ions alter properties and structure of cellular membranes, inhibit many enzymes like alkaline phosphatase, acetyl cholinesterase, and adenyl cyclase (Qitu et al., 2002,). Antagonistic interactions between Al ions and other elements such as: calcium, magnesium, iron, silicon, phosphorus, copper, and zinc were observed in biological systems (Ward et al., 2001).

Propolis has been used in folk medicine since ancient times and is known for its antimicrobial, antiparasitic, antiviral, anti-inflammatory, antitumor and antioxidant properties (Yousef , 2004;). The chemical composition of propolis is

extremely complicated, containing more than 300 components such as flavonoids, phenolic acids and their esters, alcohols, ketones, amino acids, and inorganic compounds (Simoes et al., 2004,) Flavonoids are thought to be responsible for many of its biological and pharmacological activities (Yousef & Salama, 2009). According to Giurgea *et al*. (1987), propolis contains cinnamic acid, benzoic acid and its esters, substituted phenolic acids and their esters, bee wax and caffeic acid phenethyl ester (CAPE). Some flavonoids found in propolis possess anti-inflammatory (acacetine), spasmolytic (quercetin, campherol and pectolinarigenin), antiulcer (luteolin and apigenin) and antibacterial activities (pinocembrin and galangin) (Ghisalberti, 1979). The liver is a critical organ which contains most of the accumulated metals and where toxic effects can be expected (Kurutas et al., 2009).

Royal jelly contains considerable amounts of proteins, amino acids including 8 essential amino acids (Prichard and Turner, 1985), hormone rich substance (testosterone) has been identified in extremely small quantities in royal jelly about 0.012g/g fresh weight (A), lipid, and sugars, royal jelly also contains vitamin A, C, D, and E, mineral salts are in descending order: (K, Ca, Na, Zn, Fe, Cu, and Mn.), enzymes antibiotic components. It also has an abundance of nucleic acid-DNA and RNA (Justin, 1996). Royal jelly has been determined to exhibit a variety of pharmacological activities including antitumor (Townsend et al., 1959), antimicrobial (Blum et al., 1959), vasodilative and hypotensive activities, as well as growth stimulating and infection preventing, antihypercholesterolemic and anti-inflammatory activities (Nagai and Inoue, 2004). For this reason, for more than 30 years, royal jelly has been used commercially in medical products, healthy foods and cosmetics, to a wide extent. Previously, several studies have been conducted on the antioxidant activity of royal jelly (Inoue et al., 2003).

Ascorbic acid (Vitamin C) is an essential micronutrient required for normal metabolic functioning of the body. It is an important water-soluble antioxidant in biological fluids (Carr and Frei, 1999). AA is essential for the formation of collagen and intracellular material, bone, teeth and for the healing of wounds. It helps maintain elasticity of the skin aids the absorption of iron and improves resistance to infection (Packer 1993). It can act synergistically, preventing lipid peroxidation and cell destruction (Escott-Stump & Mahan 2000). Many biochemical, clinical, and epidemiologic studies have indicated that AA may be of benefit in chronic diseases such as cardiovascular disease, cancer, and cataract, probably through antioxidant mechanisms (Carr and Frei, 1999) Humans and other primates have lost the ability to synthesise AA as a result of a mutation in the gene coding for l-gulonolactone oxidase, an enzyme required for the biosynthesis of AA via the glucuronic acid pathway. Thus, AA must be obtained through the diet (Carr and Frei, 1999).). AA is able to scavenge free radical of both reactive oxygen group (super oxide and hydro peroxyl) and reactive nitrogen group (nitrogen dioxide and peroxynitrite) (Northrop-Clewes and Thurnham, 2007).AA may be capable of regenerating other antioxidants like vitamin E therefore prevents oxidative damages (Panda et al., 2001). The idea of using AA to treat and prevent cancer was first proposed in 1949.

2. Materials and methods

2.1. Chemicals

In this study, Aluminum chloride ($AlCl_3$) was purchased from Aldrich chemical Company(Milwaukee, Wis, USA) while propolis and royal jelly were purchased from Chemist's. Vit C was obtained from Sigma. All other chemicals used in the experiment were of analytical grade. The dose of aluminum chloride ($AlCl_3$) was 34 mg $AlCl_3$/L (1/25 LD_{50}). The dose of AA, propolis and royal jelly were 50 mg/kg BW. These doses were selected based on those reported in literature.

2.2. Animals and diets:

Thirty female albino rats (Sprague-Dawely), Rattus norvegicus albinu s, with body weight of 160-170 gm were obtained from the Faculty of Agriculture, Menia University, Egypt, and were acclimated for one week prior to the experiment. They were housed in groups of five each in universal polypropylene cages at room temperature (25±2◦C) and at a photoperiod 12 h/day. Animals were fed on standard laboratory chow diet which contains 17% protein and water *ad libitum* till the end of the experiment (8 weeks). Animal experiments and housing procedures were performed in accordance to the animal care rules and they were approved by the authorities of the University.

2.3. Experimental design

The work was conducted using 30 female rats. The rats were randomly divided into five equal groups of six rats each. One of the groups served as control group while the four groups II, III, IV and V received 34 mg $AlCl_3$/ L in drinking water daily. Group II received only $AlCl_3$. Group III, IV and V were administrated orally with ascorbic acid, biopropolis and royal jelly, respectively at a dose 50 mg/kg bw twice a week. For 8 weeks. Body weight (BW) was recorded weekly during the experimental period (8 weeks) and food intake was measured daily during the adaptation and the experimental periods. At the end of the experimental period (8 weeks) animals were fasted overnight,

necropsied and blood samples were withdrawn by a fine capillary glass tubes from the orbital plexus vein. These blood samples were then collected to the non anticoagulant tubes for serum isolation. To isolate the serum, the blood was left to clot at room temperature for about 20 minutes and then centrifuged at 3000 r.p.m for 15 minutes; the supernatant serum samples were drown in dry clean-capped tubes and kept in deep freezer at –20 ^{o}C until conducting the biochemical analysis. Liver, kidney and brain organs were excised, rinsed in chilled saline solution, and then plotted on filter paper, weighed separately to calculate the absolute organs weight.

2.4. Biochemical parameters:

Liver function tests as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with colorimetric method (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) was measured calorimetrically (Belfield and Golberg, 1971). Total protein, albumin, and blood urea nitrogen (BUN) were measured according to the methods of Doumas et al., (1971) and (Fawcett and scott, (1960) respectively.

2.5. Histopathological studies:

Small pieces of liver and kidney of each animal of control and treated groups were fixed in 10% normal saline solution for twenty four hours. Washing was done using tap water then serial dilutions of absolute ethyl alcohol were used for dehydration. After routine processing, paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for histopathological examination through the light microscope. (Lillie and Fullmer, 1976).

2.6. Statistical analysis:

The data are expressed as means ± SD. Statistical analysis was performed by one-way ANOVA analysis using the MSTAT C program version 3 and means were compared using L.S.D rang according to (Gomez and Gomez, 1984).

3. Results and discussion

3.1. Changes in body weight and food consumption

The animals that were administered aluminum had significantly lower body weight when compared to controls (Table 1) ($p<0.05$), Aluminum administration had a detrimental effect on the body weights of rats, which is in agreement with previous reports (Julka et al., 1996). The lower body weight could indicate that Al treatment influences the bioavailability of some nutrients. The treated of AA, biopropolis with $AlCl_3$ increased significantly daily body weight and feed efficiency when compared to $AlCl_3$ group (Table 1) While royal jelly increased daily body weight and feed efficiency but not significant and normalized to their control values

3.2. Changes in relative organ weights:

Changes in liver, kidney and Brain weights relative to the total body weight are shown in table 2. Treating rats with $AlCl_3$ for 8 weeks increased the relative weight of liver together with kidney but significantly only in kidney when compared with those of untreated rat (control). While the brain did not illustrate any change in their relative organ weight compared to control. The harmful effect of $AlCl_3$ on the relative weight of the above mentioned organs to total body weight, has been markedly remediated with applying AA, Royal jelly, or biopropolis plus $AlCl_3$. This means that AA, Royal jelly, and biopropolis might play protective effect on aluminum toxicity in rats.

3.3. Changes in Biochemical parameters:

Several of soluble enzymes of blood serum have been considered as indicators of the hepatic dysfunction and damage. The activities of serum marker enzymes (AST, ALT and ALP) were found elevated markedly in rats treated with $AlCl_3$ (Table 3). No such changes were observed in control rat samples. The releasing transaminases (AST and ALT) from the cell cytosol can occur secondary to cellular necrosis. The activity of AST is significantly increases in such cases and escapes to the plasma from the injured hepatic cells. Also, an elevated serum level of ALT is indicative of liver disease (Gaskill et al. 2005). Viezeliene et al., (2011) found that the 4-fold increased activity of intracellular enzyme ALT in serum of mice that were treated with Al indicates significant liver cellular damage has occurred. It increases in serum when cellular degeneration or destruction occurs in this organ (Hassoun and Stohs, 1995).

Therefore, increases in the serum level of these enzymes suggests liver damage and alterations in liver function. Because it is a membrane-bound enzyme related to the transport of various metabolites, ALP is a sensitive biomarker of liver disease (Lakshmi,et al., 1991). $AlCl_3$-induced increases in ALP activity is in agreement with published results (Ochmanski. and Barabasz, 2000, Szilagyi et al., 1994) This was confirmed by histopathologic study (El- Demerdash et al., 2005) that showed

marked changes in hepatocytes as well as proliferation of duct epithelium, dilatation and congestion of blood vessels as well as mononuclear infiammatory infiltrate in rabbits treated with $AlCl_3$. Also, Wilhelm et al. (1996) reported that Al exposure can result in Al accumulation in the liver and this metal can be toxic to the hepatic tissue at high concentrations.

The presence of AA with $AlCl_3$ decreased the induction of AST, ALT, and AlP and maintained the levels of these enzymes to the normal values. Also, previous studied (Yousef et al., 2003a; Yousef, 2004) showed the protective effects of AA against the harmful effects of aflatoxin B1 and Al on the activities of theses enzymes. Also, Sies and Stahl (1995) reported that AA can protect biomembranes enzymes against peroxidative damage of xenobiotics. Ascorbic acid caused alleviation for the toxicity of $AlCl_3$ on the enzyme activities in plasma, liver, testes and brain of male rabbits treated with $AlCl_3$ (Yousef, 2004). Anane and Creppy (2001) reported that vitamins C when added in human foreskin fibroblast cultures as free radical scavenger was efficient in preventing malondialdehyde production by Al, indicating that oxidative processes are one of the main pathways whereby this metal induces cytotoxicity. Dhir et al. (1993) found that oral administration of ascorbic acid for 7 consecutive days before exposure of mice to Al reduced the frequencies of sister chromatid exchanges induced by Al.

The results indicated that treatment with $AlCl_3$ plus biopropolis could bring a significant decrease in activities of these enzymes when compared to $AlCl_3$ group. This result is in agreement with the findings that biopropolis induced reduction of the increased activity of AST and ALT in plasma of rats treated with $AlCl_3$ (Newairy et al. 2009). propolis has beneficial influences and could be able to antagonize AlCl3 toxicity (Turkez et al ,2010). This decrease supports the hepatoprotective effects of propolis. Flavonoids found in propolis may be responsible for the positive effect of propolis on these enzymes (Sanz et al. 1994). Also, biopropolis is able to induce hepatoprotective effects on paracetamol induced liver damage in mice (Nirala et al., 2008 a,b). Taken together, these findings constitute evidence that the antioxidative properties of the propolis contribute to the prevention of damage induced by $AlCl_3$ in rats. The antioxidant activities of propolis and its polyphenolic / flavonoid components are related to their ability to chelate metal ions and scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxynitrite (Ferrali et al., 1997).

While treatment the rats with $AlCl_3$ plus royal jelly the activities of AST, ALT and ALP were normalized to their control values. This effect could be attributed to the royal jelly contain vitamin C, vitamin E and arginine (Bayer, 1990). Vitamin E and C is a well-documented antioxidant and has been shown to inhibit free-radical induced damage to sensitive cell membranes of the testis and reduced lipid peroxidation in tissue estimation by malodialdehyde, so vitamin E and C significantly decreased MDA, and increased in glutathione level (Ebisch, 2006). Therefore, the present results pointed out that the treatment of AA, biopropolis and royal jelly provided protection against liver damage.

Data presented in Table 4 showed that treatment with $AlCl_3$ caused significant increase ($P<0:05$) in serum urea while the levels of total protein and albumin were significantly decreased as compared with the control. On the other hand, globulin did not change (Table 4).

The inhibitory effect of $AlCl_3$ on protein profile is in agreement with the finding of Yousef (2004) and El-Demerdash (2004). Although the intestine regulates the uptake of amino acids, the liver is of major importance because it regulates protein metabolism. So, the significant decrease in the concentrations of total proteins in rats treated with $AlCl_3$ particularly the albumin could be attributed on the one hand to an under nutrition and on the other hand to a reduction of the protein synthesis in the liver (Cherroret et al., 1995).

The elevation in serum urea level in $AlCl_3$-treated rats is considered as a significant marker of renal dysfunction (Table 4), and this result supported by the finding of Mahieu et al. (2005), who reported that alterations in serum urea may be related to metabolic disturbances (e.g. renal function, cation–anion balance). In addition, Katyal et al. (1997) reported that aluminum has been implicated in the pathogenesis of several clinical disorders, such as renal dysfunction. The increase in urea concentrations in serum of animals treated with aluminum may be due to its effect on liver function, as urea is the end-product of protein catabolism and this is confirmed by the decrease in serum proteins (Table 4) and /or referred to liver dysfunction as proven by the increase in serum AST, ALT and ALP activities (Table 3).

The present data showed that AA plus $AlCl_3$ increased the reduction in plasma proteins of animals treated with $AlCl_3$ and this is in agreement with our previous studies (Yousef et al., 2003a; Yousef, 2004). Sahin et al. (2002) reported that greater dietary vitamin C resulted in an increase in total protein and albumin concentrations in Japanese quails. Also, treatment with AA decreased serum urea and minimized the toxic effects of Al (Table 4).

Also, treatment of rats with $AlCl_3$ plus propolis decreased urea, and increased total protein levels compared to the rats treated with $AlCl_3$. This suggests that propolis can modulate protein metabolism and returned the increased level

of urea back to the control levels. These observations are similar to the data reported by Yousef (2004) In addition; Newairy et al. (2009) reported that the presence of propolis with $AlCl_3$ alleviated its toxic effects in rats treated with $AlCl_3$.

While treatment the rats with $AlCl_3$ plus royal jelly decreased serum urea, but it increased TP and albumin. However, the treatments did not cause any significant change in globulin as compared to the control. The presence of AA, biopropolis or royal jelly with $AlCl_3$ maintained the levels of all above parameters closer to the normal values (Table 4).

The histopathology of liver are shown in Fig.1, where (I) represents the normal liver of control group. Al-treated group showed hydropic degeneration of hepatocytes and sinusoidal leucocytosis (II) and portal infiltration with leucocytes (III). Liver of rat from group biopr + Al showed Kupffer cells activation and slight hydropic degeneration of hepatocytes (IV). Moreover, liver of rats from groups R+ Al and AA + Al revealed no changes with slight hydropic degeneration of hepatocytes (V and VI).

The histopathology of kidneys are shown in Fig.2, where (I) represents the kidneys of control, untreated rat which revealed the normal histological structure of renal parenchyma. On the other hand, kidneys of rat from group Al showed congestion and vacuolations of glomerular tufts (II) as well as atrophy of glomerular tufts (III). However, Kidneys of rat from group biop + Al revealed dilatation and congestion of renal blood vessel (IV), vaculations of endothelial liming glomerular tufts and epithelial liming renal tubules (V). Kidneys of rat from group R + Al showed pyknosis of the nuclei of tubular epithelium (VI). However, Kidneys of rat from group AA + Al revealed congestion of capillary tufts and intertubular blood capillaries (VII).

Conclusion

Al has adverse effects on human health. The results demonstrate that the administration of $AlCl_3$ to rats at a dose of 34 mg/L daily for a period of 8 weeks is capable of inducing marked alterations in biochemical (Albumin. total protein, AST, ALP and ALT activities/levels) parameters. The use of AA, biopropolis, or royal jelly combined with $AlCl_3$ were ascertained to alleviate the harmful effects of $AlCl_3$ in the mentioned parameters.

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Table 1. Body weight gain (g) and food consumption in rats treated with $AlCl_3$, $AlCl_3$+ AA, $AlCl_3$+biopropils and $AlCl_3$+ Royal jelly

Groups	Mean Initial wt (g)	Mean Final wt (g)	Body weight gain(g) Mean±SD	Daily body weight gain (g) Mean±SD	Daily feed intake (g) Mean	Feed efficieny Ratio(%) Mean
Control	158±16.6	235±20	$77.3±9.9^{a}$	$1.36±0.15^{a}$	14.9	9.60^{a}
$AlCl_3$	163±20.7	209±21.7	$45.8±3.5^{b}$	$0.80±0.1^{b}$	13.3	6.04^{c}
$AlCl_3$ + AA	155±20	231±16.5	$75.9±16.5^{a}$	$1.33±0.29^{a}$	13.9	9.59^{a}
$AlCl_3$+biopropels	155±15	222±10.9	$65.3±7.8^{a}$	$1.18±0.14^{a}$	13.6	8.66^{ab}
$AlCl_3$+Royal jelly	156±16	221±21.2	$64.5±7.9^{a}$	$1.13±0.12^{a}$	14.9	7.57^{bc}
LSD			14.26	0.247		1.758

Values are expressed as mean ± S.D. (n=6). The groups in the same column with different letters are statistically significant ($p < 0.05$).

Table 2. Relative organ weight* (g%) data in female rats treated with $AlCl_3$, $AlCl_3$+ AA, $AlCl_3$+biopropels and $AlCl_3$+ Royal jelly

Groups	Liver weight		Kidney weight		Brain weight	
	(g)	(%)	(g)	(%)	(g)	(%)
Control	8.69 ± 0.35^{ab}	3.55	1.37 ± 0.17^{b}	0.66	1.86 ± 0.04^{a}	0.76
$AlCl_3$	9.86 ± 1.19^{a}	4.27	1.72 ± 0.22^{a}	0.72	1.81 ± 0.03^{a}	0.90
$AlCl_3$ + AA	8.47 ± 0.91^{b}	3.63	1.28 ± 0.04^{b}	0.58	1.86 ± 0.10^{a}	0.83
$AlCl_3$+Propels	8.61 ± 0.42^{ab}	3.82	1.36 ± 0.01^{b}	0.60	1.80 ± 0.04^{a}	0.80
$AlCl_3$+Royal jelly	8.86 ± 1.94^{ab}	3.91	1.40 ± 0.18^{b}	0.62	1.90 ± 0.16^{a}	0.84
LSD	1.304		1.168		1.179	

Values are expressed as mean ± S.D. (n=6). * Organ weight/body weight × 100. *The groups in the same column with different letters are statistically significant ($p < 0.05$).*

Table 3. changes in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of female rats treated with $AlCl_3$, $AlCl_3$+ AA, $AlCl_3$+biopropels and $AlCl_3$+ Royal jelly

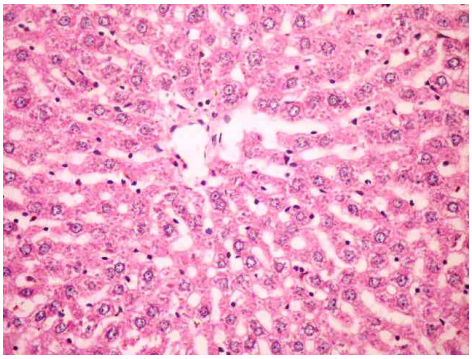
Groups	ALT (U/L)	AST (U/L)	ALP (IU/L)
Control	$15.9\pm.506^{b}$	41.7 ± 1.86^{b}	71.08 ± 2.03^{b}
$AlCl_3$	25.6 ± 2.65^{a}	106.3 ± 4.17^{a}	105.3 ± 2.36^{a}
$AlCl_3$ + AA	$16.3\pm.558^{b}$	46.4 ± 1.86^{b}	75.3 ± 2.12^{b}
$AlCl_3$+biopropels	15.8 ± 1.55^{b}	37.2 ± 1.54^{c}	76.2 ± 1.37^{b}
$AlCl_3$+Royal jelly	$16.8\pm.814^{b}$	$47.7\pm.706^{b}$	74.99 ± 1.63^{b}
$LSDat_{0.05}$	1.213	7.116	10.71

Values are expressed as mean ± S.D. (n=6). The groups in the same column with different letters are statistically significant ($p < 0.05$).

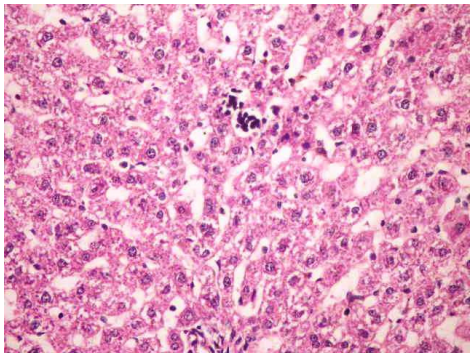
Table 4. changes in concentration of total protein, albumin, globulin and blood urea nitrogen (BUN) in serum of female albino rats of rats treated with $AlCl_3$, $AlCl_3$+ AA, $AlCl_3$+biopropels and $AlCl_3$+ Royal jelly

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	BUN (mg/dl)
Control	7.13 ± 0.22^{a}	4.57 ± 0.57^{a}	2.59 ± 0.32^{ab}	35.8 ± 1.37^{c}
$AlCl_3$	5.87 ± 0.12^{b}	3.86 ± 0.49^{b}	2.01 ± 0.13^{b}	73.0 ± 0.59^{a}
$AlCl_3$ + AA	6.87 ± 0.36^{a}	4.42 ± 0.63^{ab}	$2..43\pm0.24^{ab}$	48.2 ± 2.02^{bc}
$AlCl_3$+biopropels	6.79 ± 0.34^{a}	4.37 ± 0.59^{ab}	2.52 ± 0.52^{ab}	48.5 ± 2.75^{bc}
$AlCl_3$+Royal jelly	6.95 ± 0.18^{a}	4.81 ± 0.46^{a}	$2.90\pm0.0.09^{a}$	54.2 ± 0.47^{b}
LSD $at_{0.05}$	0.408	0.6143	0.731	14.59

Values are expressed as mean ± S.D. (n=6). The groups in the same column with different letters are statistically significant ($p < 0.05$).



(I)



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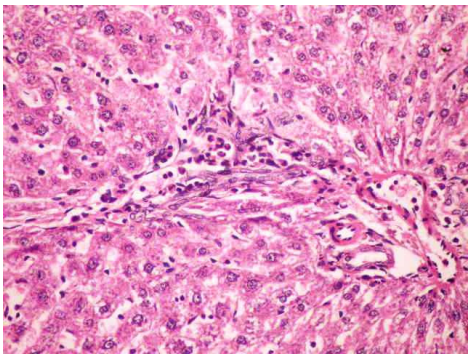

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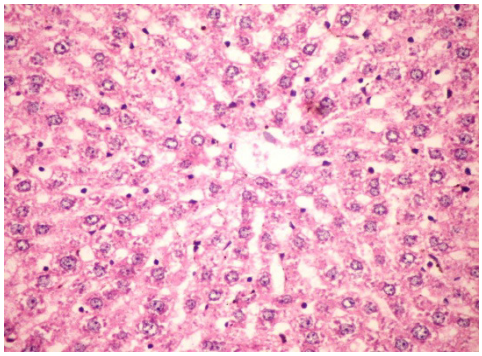

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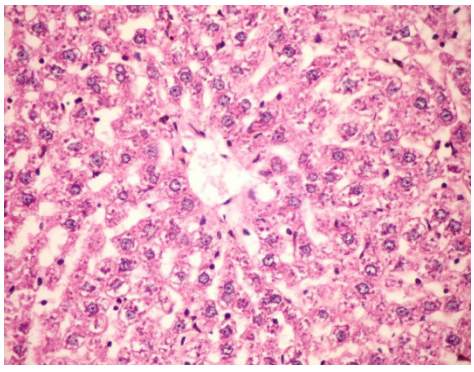

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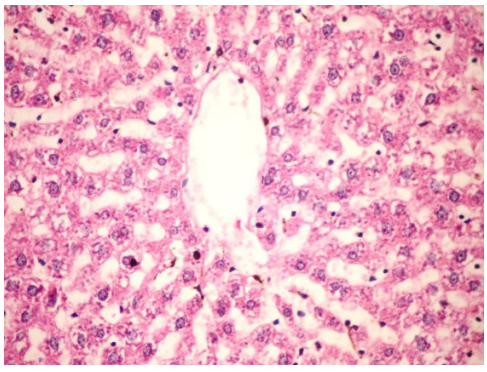

(VI)

Fig.1. liver histology of rats from untreated control group (I), group with hydropic degeneration of hepatocytes and sinusoidal leucocytes (II), group received Aluminum shows portal infiltration with leucocytes (III), group received Bropolis and Aluminum shows Kuppffer cells activation with slight hydropic degeneration of hepatocytes (IV), group received Royal gel and Aluminum shows slight hydropic degeneration of hepatocytes (V) and group received Ascorbic Acid and Aluminum shows slight hydropic degeneration of hepatocytes (VI).

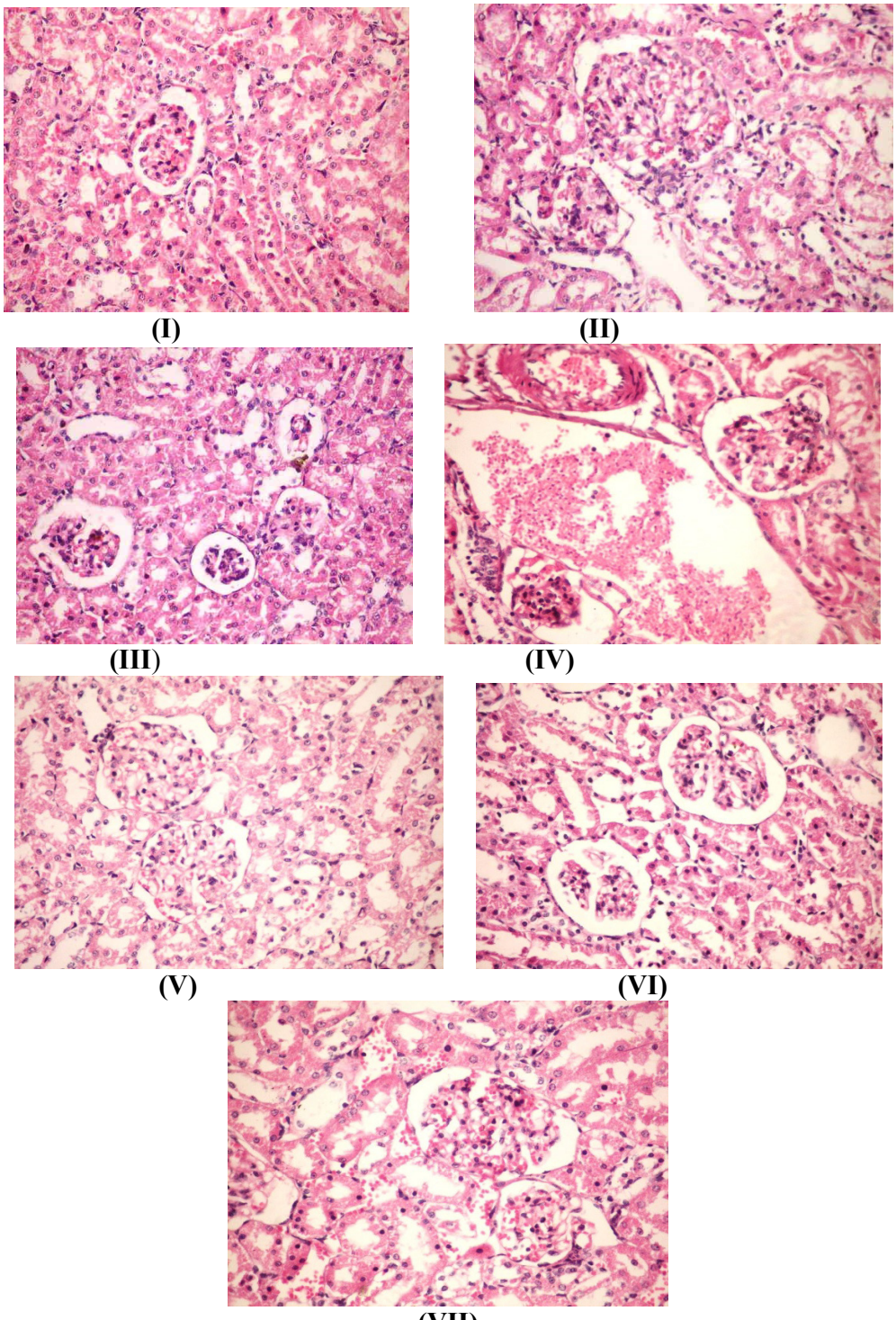

Fig. 2. Kidney histopathology of control, untreated rat (I). Kidney of rat treated with Aluminum (II) and (III); Kidney of rat received Bropolis + Aluminum group shows dilatation and congestion of renal blood vessel (IV):; Kidney of rat received Bropolis + Aluminum group shows vacuolations of endothelial lining glomerular tufts and epithelial lining renal tubules (V); Kidney of rat group received Royal gel and Aluminum shows pyknosis of the nuclei of tubular epithelium (VI); .Kidney of rat received Ascorbic Acid + Aluminum group shows congestion of capillary tufts and intertubular blood capillaries (VII):. All Fig H and E X 400

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