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ASSESSMENT OF THE AMELIORATIVE ROLE OF PROPLIS AND MALIC ACID IN INTESTINAL AND LIVER FUNCTIONS OF ALUMINUM EXPOSED MALE RATS

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ABSTRACT
The comparative effect of propolis and malic acid in reducing the effects of oral aluminum chloride administration on intestinal and liver functions of adult male rats were studied. Forty adult male rats were divided randomly into four equal groups ; first group was a control group (C group) ;animals of 2nd group received AlCl3 (50 mg /kg body weight) (AL group), animals of 3rd group received wAlCl3 (50 mg /kg body weight)+ propolis (50 mg /kg body weight) (ALP group) ; the animals of the 4th group administered with AlCl3 (50 mg /kg body weight) + Malic acid (45 mg /kg body weight) (ALM group).The experiment lasted for 60 days rats of different groups received the above materials through a stomach tube. Results of the present study showed that liver function enzyme was significantly elevated by $AlCl_3$ adiminstration for 60 days. The combination of ALCl3 plus propolis or malic acid reduced this significant elevation to a semi normal value. On the other hand examination of liver and intestinal H&E stained sections showed the negative and deleterious effects of aluminum, these effects were improved in rats received propolis or malic acid.

KEY WORDS: Aluminum chloride, propolis, malic acid, liver enzyme, intestinal mucosa.

INTRODUCTION

Various environmental chemicals, industrial pollutants and food additives have been implicated as causing harmful effects. 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 [1,2,3,4]. Al is widely distributed in the environment and extensively used in daily life, which causes its easy exposure to human beings. It gets access to the human and animal's body via the gastrointestinal and the respiratory tracts. Al accumulates in all tissues of the mammals, including kidney, liver, heart, blood, bone and brain [5,6]. Propolis, a resinous wax-like beehive product is collected by honey bees from plant exudates and also known as bee glue. The worker bees apply the resin to seal any cracks and fissures in the hive and they 'line their front door' with it to prevent contamination. They use it as an antiseptic in breeder cells, and they mix propolis with wax to distribute a fine varnish over every inch of the hive to protect it. Chemical properties of propolis are not only beneficial to bees but have general pharmacological value as a natural mixture [7]. Several empirical and clinical findings point to the fact that propolis may be more effective against pathogenic microorganisms and environmental pollutes like Lead [6,8]. The propolis has been used in folk medicine for antioxidant, immune-stimulating, anti - inflammatory and non - toxic natures [9] Propolis has also been found to have powerful anti-inflammatory properties [10]. Propolis can counteract the damaging effects of aluminum, Aluminum provoked nephrotoxicity [11], cardiotoxicity [1] hepatotoxicity, hematotoxicity [13] and neurotoxicity [14], Besides, Al caused genetic damage in rat bone marrow cells[1]. Malic Acid has good chelating properties, chelating agents defined as a "chemicals that form soluble, complex molecules with certain metal ions such as aluminum, cadmium, lead, mercury, arsenic, inactivating the ions so that they cannot normally react with other elements or ions to produce precipitates or scale [16] Malic Acid is one of the most potent chelators of aluminum and was the most effective of several chelators tested at reducing aluminum levels in the brain [17, 18]. Treatment with Malic Acid has been shown to greatly increase the fecal and urinary excretion of aluminum and reduce the concentration of aluminum found in various organs and tissues [19] and plants [20].The aim of the present study was to evaluate the possible ameliorative role of proplis or malic acid against negative effects of aluminium chloride exposure could have on the functions of small intestine and liver of wistar rats.

MATERIAL & METHODS

Experimental Animals

A total of forty Male Albino Wister rats were at age 8 – 9 weeks and their body weight ranged between 100 – 120 grams, were kept in the same suitable environmental conditions of 22 – 27 C°, and photoperiod of 12 hours daily. After two weeks of adaptation, rats were divided randomly into four groups and each group contained ten animals; Group (C): Control group orally administered distil water, Group (AL): Aluminum group orally administered AlCl3 (50 mg /kg body weight), dissolved in distil water daily Group (ALP) : Aluminum + Propolis group orally administered AlCl3 (50 mg /kg body weight) dissolved in distil water and propolis (50 mg /kg body weight) , daily Group (ALM) : Aluminum + Malic group acid orally administered AlCl3 (50 mg /kg body weight)

dissolved in distil water and Malic acid (45 mg /kg body weight) dissolved in distil water. All applications were administered daily for 60 consecutive days. At The end of the experiment blood sample (4-5 ml) was collected from the rat obtained via cardiac puncture technique from each anesthetized animal using disposable syringe (5 ml) and blood was withdrawn into plastic test tubes (gel tube) for serum isolation for biochemical analysis.

Biochemical analysis

Determination of serum alanine aminotransferase (ALAT)

ALAT activity was determined colorimetrically**.** The color absorbance was obtained by coupling of pyruvic acid and L-Glutamic acid with 2, 4-Dinitrophenylhydrazine. The corresponding colored hydrazones was measured at wave length of 546 nm.

Determination of serum aspartate amino transferase (ASAT)

Serum is incubated with ketoglutarate for one hour at 37°C and the reaction is stopped and dinitrophenylhydrazine was added. The color absorbs light at 505 nm.

Determination of serum alkaline phosphatase (ALP)

Alkaline phosphatase, in alkaline medium, hydrolyzes a colorless substrate of disodium phenyl phosphate giving rise to phenol and phosphate.4 aminoantipyrine and sodium arsenate are used to stop the enzymatic reaction. The liberated phenol could then be measured colorimetrically by adding potassium ferricyanide as a color developing reagent.

Histopathological changes

Liver tissues preserved in 10%neutral formalin buffer solution,after fixation, the tissue was trimmed and the specimen were washed with saline for (1-2hrs) and transferred to following steps:1. Dehydration: Specimen was passed through ascending grades of ethanol alcohol (70%, 80%, 90%, 100%). For 1 hour in each concentration 2. Clearing: Two solutions of xylol commonly used for clearing. The specimens rested 1 hour in each step 3. Impregnation with Paraffin wax 4. Blocking 5. Sectioning6.Staining with Prussian blue stain for hemosidrine.

Statistical analysis

Data are shown as the Mean± SE (stander error) when a significant interaction between major factors was identified by ANOVA SPSS version 11, LSD test was used post-ANOVA to identify significant differences between mean values at probability level of ($p<0.05$) was taken as significant

RESULTS & DISCUSSION

The ameliorative role of proplis and malic acid in liver function enzymes of Aluminum chloride exposed male rats for 60 days represented in table-1. The results of the present experiment showed that Aluminum chloride caused significant increase($p<0.0001$) in level of liver enzymes; ALP, ALT, and AST (28.17±2.5,4± 4.8,7.7, 259.83±.8) when compared with control group (6.30±.8,9.70±.9,23.71±.3) this increase was modified significantly in the group received Aluminiumchloride +propolis (7.01±.2,17.01±.9,38.801±1.3) and in the group which received Aluminium chloride + malic acid (10.73 ± 0.8, 22.8 ± 3.6, 133.2 ± 8.6) , to asemi normal values , respectively. The role of Aluminum in causing liver damage is receiving considerable attention, because Liver plays an important role in the organization of many metabolic activities and manufacturing of many important materials inside the body. Serum liver function enzymes including AST,ALT, and ALP showed increased activities after long term administration of Al, these results are in accordance with [21,22] who found an increased release of the enzymes AST and ALT due to high dose of Al. this suggest that chronic Al exposure induce hapatoxicity manifested by elevation of liver function enzymes. The presence of liver disease often is recognized on the basis of elevated serum activities of enzymes of hepatic origin alanine aminotransferase (ALT), aspartate aminotransferase (AST) [23]. Although they are sometimes referred to as "liver function tests," serum enzymes do not measure hepatic function directly but indicate alteration in the integrity of the cell membrane of the hepatocyte, necrosis of hepatocytes or biliary epithelium, impeded bile formation or bile flow (cholestasis), or the induction of enzyme synthesis [24] Serum AP activity may be elevated in acute and chronic liver diseases. More marked elevations indicate cholestasis, with the highest serum AP activities observed in animals with cholangitis, biliary cirrhosis, or extrahepatic bile duct obstruction. Following the experimental production of hepatic necrosis in following bile duct obstruction, the serum activity of both AP and GGT increases remarkably along with moderate elevations in ALT and AST.

TABLE-1: Assessment of the ameliorative role of proplis and malic acid serum level of liver function enzymes ALT (U/l) AST (U/L) and ALP (U/L).

Experimental groups	ALT	AST	ALP
Control group (C)* (Distil Water)	6.3±0.8 c	9.7±0.9 c	23.71±.3 c
Aluminum chloride (AL)* (50 mg /kg B.W.)	28.172±.5 a	44.87.±7 a	259.8 3±.8 a
Aluminum chloride* & propolis (AP) (50 mg/kg B.W.)	10.73±0.8 b	22.83±.6 b	133.28±.6 b
Aluminum chloride* & Malic acid (AM) (45 mg/kg B.W.)	7.01±.2 c	17.01±.9 cb	38.81±1.3 c

Small superscript denote significant ($p<0.05$) difference between groups (Column).

* Orally administration

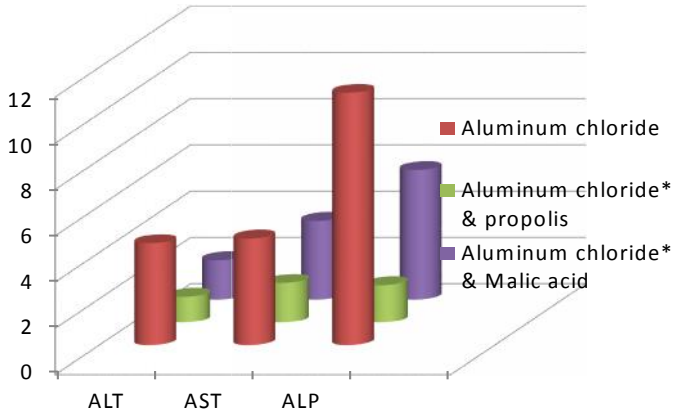

Figure -1: Assessment of the ameliorative role of proplis and malic acid serum level of liver function enzymes ALT (U/L) AST (U/L) and ALP (U/L) as no of folds from control.

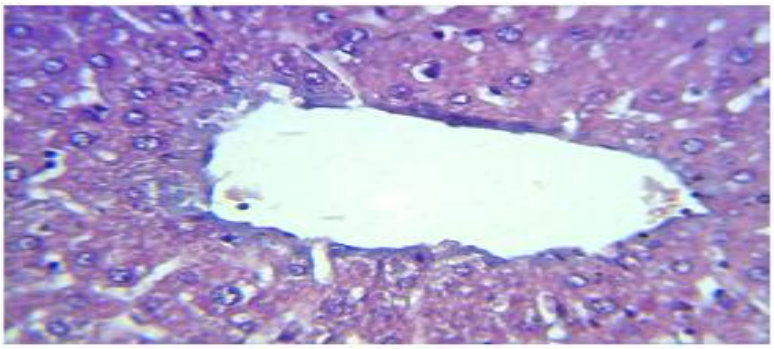

Fig-2 :-Histological section of the liver of wistar rats of control group showing the central vein and heptocytes of hepatic cord with radially arranged sinusoids .X400 H&E stain.

The significant elevation of liver function enzymes denoted in the present experiment represented in figure-1 as folded from normal indicate the great cellular damage of hepatocytes caused by aluminum administration. In the current study the increased in total Serum liver function enzymes including AST, ALT, and ALP levels in rats administered AlCl3 (table -1), could be attributed to increased in the damage of the liver tissue [22]. Aluminum chloride enhance the production of free radicals accelerating the oxidative stress effects on hepatocellular membrane[23] in resulting leakage of these enzymes in to the blood stream higher than normal condition. Al alters biochemical parameters induces oxidative stress and histopathological alterations[21] .The result revealed that oral administration of propolis or malic acid caused a significantly depression in serum liver function enzymes including AST, ALT, and ALP levels concentration as a compared to control group. Propolis it can play a prevention role in the free radical reaction [27]. It has been found that administration of propolis decreased lipid peroxidation incellular membrane [28].

The chelating & antioxidant properties of malic acid have been well documented precisely[29,20]. There for administration of malic acid protect hepatic tissue from deleterious effect of AlCl3[19]. Accordingly, the antioxidant activity of both propolis and malic acid and chelating activity of malic acid restore cellular integrity with maintaining liver function including maintaining AST, ALT, and ALP concentration. The present results are in agree with[30] who found that honey (antioxidant) exhibit a protective potential by improving the disturpted liver biochemical marker, that all alleviating the increase lipid peroxidation induce by Aluminium chloride , in addition effect is due to ability counteract the oxidative damage and protect liver tissue and restore the normal metabolic process. On the other hand malic acidis known for its ability to increase energy (ATP) because it is an essential component in Krebs cycle and that (malic acid) consider as antioxidant [31]. Thus, treatment with propolis or malic acid could improve cellular membrane & organ functioning more profoundly and brought all these variables in liver function enzymes activity to ward control group. Therefore, both compounds can alleviate the Al-mediated hapatoxicty in rats.

The results of the present study have shown that light microscopic evaluation of intestinal wall and liver tissues sections revealed the negative and deleterious effects had Aluminum chloride on the treated groups when compared with the control and propolis or malic acid received groups. Liver tissue section of control group showed normal histological structure the central vein and heptocytes of hepatic cord with radially arranged sinusoids (fiqure-2), while in asecond group which received Aluminum chloride (fiqure-3) showed vascular

degeneration of hepatocytes containing small nuclei with presence of prominent nucleoli .there are many large necrotic area with blood oozing to the necrotic area .many section showed a formation of early granuloma with the infiltration of mononuclear cells and a few fibroblasts in the portal area around the bile ductules and portal blood vessels .in addition to the previous histopathological changes there is severe diltation and congestion of centeral veins ,sinus and portal blood vessels. While in group that received Aluminium chloride +propolis the hepatic parenchyma exhibited microscopic finding resemble that of control with profiliration of kuffer cells and slight congestion of blood vessels (fiqure-4). Liver tissue section of rats received Aluminium +malic acid showed similar histopathological changes to control group with focal aggregation of mononuclear cells (fiqure-5) . The liver is a critical organ which contains most of the accumulated metals and where toxic effects can be expected [32, 33] stated that aluminium treated wistar rats showed distortion of the arrangement of parenchyma of the liver, loss of radial arrangement of sinusoids from the central vein of the liver and loss of hexagonal shape of the hepatocytes when compared with the control. Based on our histological observations, we therefore conclude that aluminium chloride exposure was detrimental to the liver of adult wistar rats and hence caution should be taken in its usage.on the other hand propolis improved these deleterious effects and prevent their occurance this may be due to the antioxidants effects of propolis preventing tissue damage due to the malic acid is one of most potent chelator of aluminium and was the most effective of several chelator tested at reducing Aluminium level in various organ especially brain and tissues [34,37]. Because the propolis as antioxidant [6,7,11,35] have been instrumental in avoiding oxidative stress on the tissue [36,38].

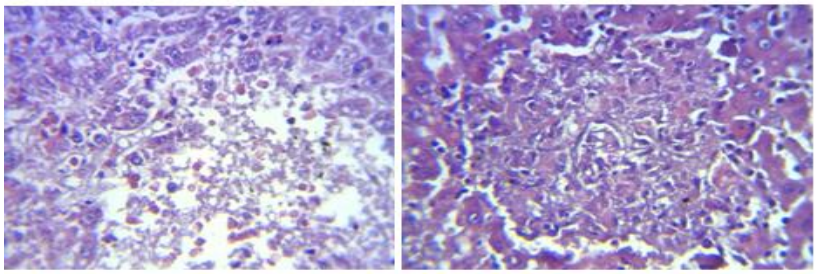

Fig-3 Histological section of the liver of waster rats group received Aluminium chloride showing: A – Large, diffuse necrotic area with cellular depress blood oozing to the necrotic area, with vascular degeneration of hepatocytes. B– focal granulomatous lesion in liver parenchyma with the infiltration of mononuclear cells and few fibroblasts, and dilatation of bile ducts assosiated with mononuclear cells aggregation and congestion of central veins ,sinus and portal blood vessels. X400 H&E stain.

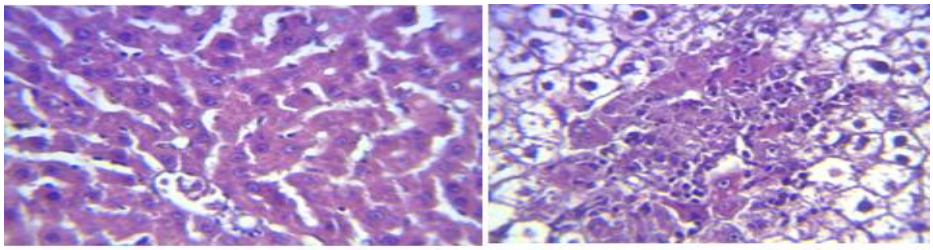

Fig-4 Histological section of the liver of wistar rats of group received Aluminium chloride+ Propolis :- showing hepatic parenchyma exhibited microscopic finding resemble that of control with profiliration of kuffer cells . X40 H&E stain :

Fig-5 Histology of the liver of wistar rats of group received Aluminium chloride+ Malic acid showing massive vaculating of epatic cells acompained with focal aggregation of mononuclear cells mainly lymphocytes and macrophages X40 H&E stain :

Intestine wall tissues section of control group showed normal structure (fig.8)resembled by goblet cells , no or little mucus production, normal lymphoid tissue with no hyperplasia, and no congestion.

Results of the histopathological intestinal tissues sections of aluminum chloride adimimstrated rats (figure-7) showed an increase in goblet cells with extensive mucus production in addition to focal infilteration of mono nuclear cells in the lumina propria of mucosa and submucosal lympfoied tissue hyper plasia. In addition, there ewre submucosal thickening with congestion and thickening of blood vessels.

The main reason for such intestinal tissue histopathological changes caused by AL could be attributed to it's capability to release free radicals in different organs this has been recently emphazied with observation of [25,28]. Consencountly aluminium chloride contributing in gastro intestinal and hepato toxicity cause inhibition in absorbtion and alter in structure of tissue [37] .

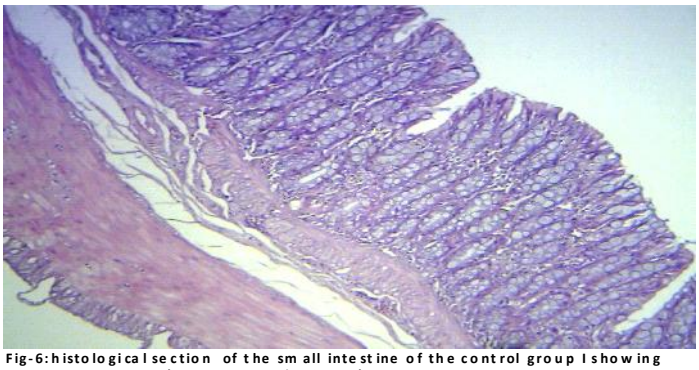

Fig-6: histological section of the small intestine of the control group I showing normal submucosa(with brunner's glands), mucosa and tall cylindrical villi with goblet cells. X40 H& E.

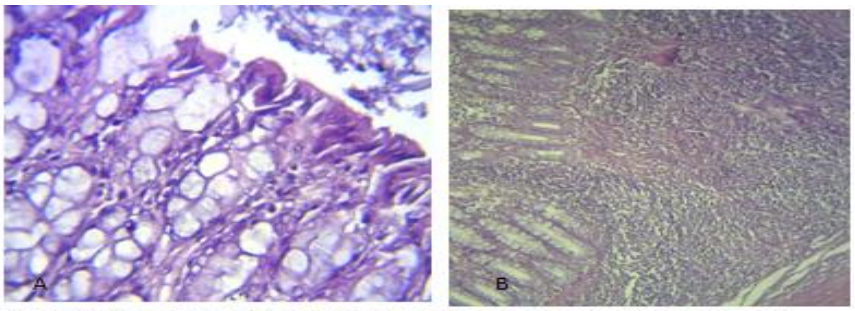

Fig-7: Histological section of the small intestine of the aluminum chloride adiministerded waster rats: showing A – an increase in number of goblet cells associated with slughing of intestinal mucosa with extensive mucus production X400, H&E . B-focal infilteration of mono nuclear cells in the lumina propria of mucosa with cellular infiltration in the blood vessels of mucosa , submucosal lympfoied tissue hyper plasia, submucosal thickening with congestion and thickening of blood vessels . X100, H&E.

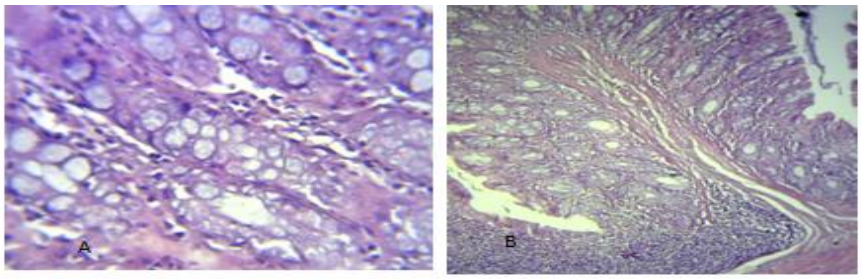

Fig-8: Histological section of the small intestine of the aluminum chloride + propolis adiministerded waster ratsshowing : A moderate increase in mononucleated cells and moderate hyperplasia of goblet cells in submucosa X400 H&E. . B- No clear pathological changes Little thickness of submucosal, focal infilteration of mono nuclear cells in the lumina propria of mucosa submucosal lympfoied tissue hyper plasia. X100 H&E.

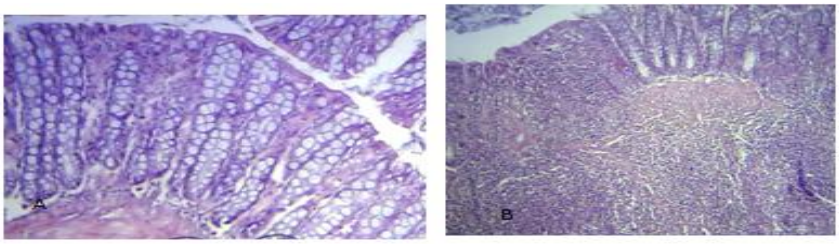

Fig-9: histological section of the small intestine of the aluminum chloride + Malic acid adiministerded waster rats showing : A – mucosa with an increase in goblet cells with few monocytes infiltration Little thickness of submucosal , X400 H&E. B- Marked cellular aggregation with fibroplasia of submucosamuscularis increase in blood vessels supply, C- sub mucosal lymphoid tissue hyperplasia. X10 0H&E.

While in group treated with Aluminum +propolis the intestinal histopathological sections revealed a moderate increase in mononononucleated cells with moderate goblet cells hyperplasia of lymphoid tissue and eosinophilia

(fiqure-8). These changes reflect the protective role of propolis against AlCl3 bad effects ,because the honey act as antioxidant, antimicrobial, antifungal and that can counteract the damaging effect of ALCL3 (Tatli seven *et al*., 2009). In addition the group that treated with (Aluminium chloride + malic acid) showed similar to control group in structure because the malic acid protects against the toxic of ALCL3 (Kabouj, 2007). Intestinal tissues sections of aluminum+malic acid group showed little submucosal thickening and lymphoied hyperplasia , with intense mononuclear infiltration (fig-9). In conclusion, results obtained from the present study score the negative effects of AlCl3 on liver and intestinal physiologically and morphologically, besides administration of propolis was effective in reducing these changes in manner better than did the malic acid.

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