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Evaluation various doses of apricot kernels effect on antioxidant system and hepatic tissue in female albino rats.

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Abstract

This study aimed to evaluate the effect of various doses of local apricot kernel on architecture of hepatic tissue and oxidant-antioxidant balance in serum and hepatic tissue. Twenty-four female albino rats were distributed into four groups randomly. First group administrated with distill water, second, third and fourth groups administrated with apricot kernel powder AKP 1.5g/kg, 3g/kg and 6g/kg respectively. Results revealed significant elevating in GSH and GPx in serum and GSH, SOD and CAT in hepatic tissue in groups administrated with 3 and 6 g/kg of APK. Moreover no alteration in MDA observed in all groups in compare to control. An elevation noticed in level of AST in administrated groups while no alteration in ALP. Histopathological study revealed a medium enlargement in hepatocyte with infiltration in a group treated with 3g/kg while there were a hypertrophy in hepatocyte, infiltration and congestion in a group administrated with 6 g/kg. We concluded that administration with 6g/kg APK may increase risk of liver cell injury, 3g/kg APK was less effect and 1.5 g/kg APK shows no alteration in compare to control.

Keywords: Apricot kernel**;** antioxidant**;** amygdalin, cyanogenic glycoside.

Introduction

Despite of the unapproved of amygdalin by food and drug agency FDA as a medicinal treatment, it used broadly for treating many diseases including anti-cancer therapy. Apricot seed has been used traditionally to relieve fever and stop coughing. It used for treating asthma, bronchitis, emphysema, constipation, nausea, leprosy, and blanching of the skin [1]. Apricot kernel oil is used in cosmetics and as a pharmaceutical laxative and expectorant agent [2]. Apricot seeds contain a well-known chemical compound such as Amygdalin (D-mandelonitrile-β-D-glucosido-6-β-D-glucoside) a naturally occurring cyanogen glycoside known as Vitamin B 17 [3]. Amygdalin degraded by enteral β-glucosidase into gentiobiose and L-Mandelonitrile. Gentiobiose hydrolyses to glucose while L-mandelonitrile hydrolyses to hydrogen cyanide and benzyldehyde. Scientific researches provides some evidence about negative effects may occur if person consumed a lot of kernels in one day and another administrated it as anti-cancer due its ability to hydrolyze to hydrogen cyanide , benzaldehyde and glucose [4]. According to the World Health Organization, the tolerable intake of cyanide is 12-20 μg/kg per day and the lethal dose started between 0.5 – 3.5 mg/kg per day [5]. This study aimed to elucidate the effect of high, medium and low doses of apricot kernels on hepatic tissue and antioxidant balance in normal rats.

Materials & Methods

Preparation of apricot kernel

Apricot kernels samples collected from local groves in Diyala Province / Iraq, during the fruit-ripening season. The kernels extracted then dried at room temperature, crushed and powdered then kept in dark glass bottles until use.

Phytochemical analysis

Cyanogenic glycosides extraction procedure:

1 gram of dried seeds extracted with 60 ml hexane to remove fats, oils, terpenes, phenols and other compounds, then added to 50ml of methanol then evaporated to 2 ml. Many cyanogenic glycoside can be

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isolated directly from the alcoholic extract by High Purified liquid Chromatography HPLC method via acidified methanol and water.

Separation condition:

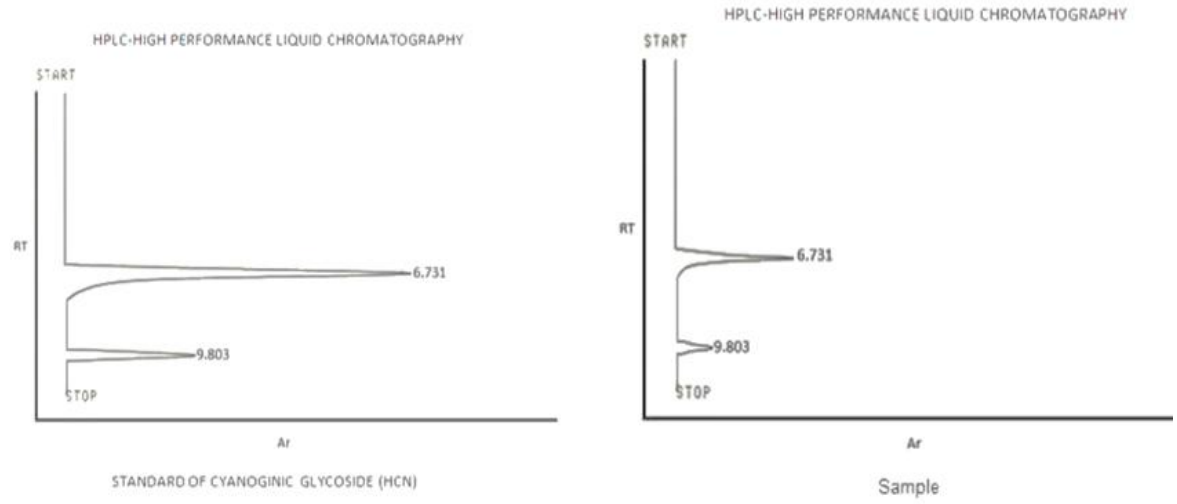
Mobile phase 40:40:20 acetonitrile: Water: methanol v/v/v, Separation of cyanogenic glycoside (HCN) mixture reversed phase C-18 (150 4.6 mm ID) column, 5um particle size ID. Flow rate 1 ml/min, experiment temperature 30 C^{o}, UV detector 215 nm. , Standard 50 um /ml.

Cyanogenic glycoside compounds: Amygdaline (HCN), amygdaline amide (HCN).

Equipment description: Shimadzu HPLC-10, double pump model HPLC-10A, japan, reodyne 7125 injector equipped with 20µl injection loop, UV detector set at 215 nm.

Calculations: Concentration = area in sample / area in standard * standard Concentration. * Dilution Percentage %= concentration partial / concentration total

Dilution for all specimens = 2 [6].



Experimental design

Twenty-four female Sprague-Dawley rats weighing 200-210 g., aged 10-12 weeks obtained and housed at the animal house unit, college of veterinary medicine, university of Tikrit, Iraq. Rats underwent to the standard laboratory conditions and fed with standard diet and water *ad libitum*. Rats distributed equally into four groups, first considered control group administrated with saline solution, second group administrated with low dose of AKP 1.5 g/kg (i.e. 42.126 mg/kg of amygdalin). Third group administrated with medium dose 3g/kg AKP (i.e. 84.252 mg/kg of amygdalin), fourth group administrated with high dose of AKP 6 g/kg (i.e. 168.504mg/kg of amygdalin). The doses elected based on the referenced lethal dose by [7].

For cyanide which it is between 2.13 and 6 mg/kg body weight. Each 1 g of amygdalin released 59mg of cyanide [8].

Blood samples collecting : After 30 days, rats starved for 12 hour then anesthetized to collect blood from jugular vein. The blood centrifuged at 3000xg for 15 min. to obtain serum. Serum kept in deep freeze at – 80 C^{o} until use.

Hepatic tissue studies

Physiological study preparation: liver tissue removed immediately, weighed and placed in cooling homogenization medium consist of 0.25M sucrose , 1mM EDTA and 20mM Tris-HCl buffer (pH 7.5) 10ml per gram of tissue. One gram of liver tissue homogenate by homogenizer (SHM1)16000 rpm for 30-60 second then placed in cooling centrifuge (-4C) 3000 rpm for 5 minutes. The supernatant used to determine antioxidant parameters and oxidative stress in liver tissue.

Histological study preparation: Liver excised and fixed in formalin 10% for 24 hour then washed with water for 30 minutes. Samples dehydrated using alcohol in gradient ascending series concentrations (70%, 80%,

90% 100%), 30 minutes for each gradient. Sample cleared by xylene for 30 min. then embedded in paraffin for 30 min. in temperature 56-58 C°. Tissue sectioned in five μm for the histopathological study. Sections dewaxed with xylene for 15-30 min., and then exposed to descending gradient concentrations of alcohol (100%, 95%, 70%, 50%, and 30%) five min. for each concentration. Sections stained with hemotoxylin stain for 7 min. at room temperature after 15 min sections stained with eosin stain for 2 min , dehydrated with ascending gradient concentrations of alcohol (70%, 80%, 96%, 100%) 2 min for each gradient then washed with xylene . sections dried and observed under microscope.

Measurements of Oxidant – antioxidant parameters: Lipid peroxidation measured via malondialdehyde MDA that make a complex with thiobituric acid gives pink color can be absorbed at 532 nm. Glutathione GSH proceed according to Ellamn method [9] , the sulfhydryl group interact with German reagent in a alkaline medium to form sulfured compound and TNB the complex gives a yellow color absorbed at 412nm..The superoxide dismutase SOD evaluated depending to degradation of the nitro blue tetrazolium by the superoxide radical. Resulted color absorbed at 560nm. Glutathione peroxidase GPx determined by measuring NADPH oxidation rate using hydrogen peroxide as a substrate, resulted color absorbed at 340nm.

Determination of liver function enzyme : Serum AST and ALT activities were measured using Randox diagnostic kits based on the method described by [10]. ALP activity measured based on [11].

Statistical analysis: Data analyzed by one – way ANOVA independent sample tests. The differences among groups achieved using Duncan multiple range test [12].

Results

Apricot kernels have two cyanogenic glycosides, amygdalin and amygdalin amide. HPLC analysis detected the concentration of amygdalin (HCN) and amygdalin amide (HCN) as shown in table (1).

cyanogenic glycoside	Retention time	Peak area	Concentration mg/g
Amygdalin (HCN)	6.731	2313	24.311
Amygdalin amide(HCN)	9.803	0357	3.752
			Total 28.063

Table 1: Detecting cyanogenic glycoside (HCN) in apricot kernels using HPLC method (table shows: retention time, peak area and HCN concentration).

The results showed (table 2) non-significant difference (P≤0.05) in the level of MDA in the serum of rats administrated with apricot seed kernels powder in concentration of 1.5, 3 and 6 grams per kg of body weight, respectively. In addition, no significant differences (P≤0.05) observed in the level of SOD, CAT in serum compared to control group, while there were a significant increase (P≤0.05) in the level of GSH and GPx in serum compared to the control group in rats administrated with 6 g per kg. Rats administrated with 1.5 and 3 grams per kg did not show a significant difference (P≤0.05) compared to the control group.

Parameters	Control	Kernel 1.5 g/kg	Kernel 3 g/kg	Kernel 6 g/kg
MDA mmol/l	1.358±0.021	1.305±0.063 N.S	1.387±0.042 N.S	1.304±0.058 N.S
GSH mmol/l	0.431±0.006	0.458±0.013 N.S	0.472±0.01 *	0.487±0.008 *
SOD IU/l	0.751±0.038	0.708±0.011 N.S	0.735±0.01 N.S	0.736±0.017 N.S
CAT IU/l	1.532±0.081	1.508±0.049 N.S	1.591±0.07 N.S	1.521±0.090 N.S
GPX IU/l	5.423±0.082	5.781 ±0.112 N.S	5.783 ± 0.094 N.S	5.978±0.071 *

Table 2: Oxidant-antioxidant parameters level in serum

- Values represented by mean ± SD. N=24.
- "N.S" means no significant difference in value P≤0.05.
- "*" means a significant difference in value P≤0.05.

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- Administrated groups compared to control.

The results of homogenized liver tissue showed no changes in MDA level of rats administrated with apricot kernels powder 1.5, 3 and 6 grams per kg of body weight, respectively, compared with the control group, while a significant increase (P≤0.05) observed in the level of SOD in rats administrated with 3 and 6 gram per kg. No significant changes observed in SOD when rats administrated with 1.5 g per kg. The results also showed a significant increase (P≤0.05) in the level of CAT in rats administrated with 1.5 and 6 g per kg, respectively, while no significant differences found when rats administrated with 3 g per kg compared to control. A significant decrease (P≤0.05) observed in the level of GSH when rats administrated with 3 and 6 grams per kg, respectively, while there were no significant differences when rats administrated with 1.5 grams per kg compared to the control (Table 3).

Parameters	control	Kernel 1.5 g/kg	Kernel 3 g/kg	Kernel 6 g/kg
MDA mmol/g	1.776±0.063	1.65±0.04 $^{N.S}$	1.593±0.039 $^{N.S}$	1.723±0.034 $^{N.S}$
GSH mmol/g	0.573±0.006	0.594±0.011 $^{N.S}$	0.505±0.001 *	0.538±0.009 *
SOD IU/g	0.976±0.013	1.056±0.025 $^{N.S}$	1.160±0.013 *	1.123±0.070 *
CAT IU/g	1.510±0.036	1.866±0.024 *	1.703±0.04 $^{N.S}$	1.910±0.074 *

Table 3: Oxidant-antioxidant parameters level in homogenized liver tissue

- Values represented by mean ± SD. N=24.
- "N.S" means no significant difference in value P≤0.05.
- "*" means a significant difference in value P≤0.05.
- Administrated groups compared to control.

Liver function enzymes showed a significant increase (P≤0.05) in the level of AST in all administrated groups, while no significant differences observed in the level of ALT compared to the control group. Table 4.

Liver enzymes	control	Kernel 1.5 g/kg	Kernel 3 g/kg	Kernel 6 g/kg
AST U/L	20.06 ±1.797	28.22±1.690 *	34.7 ± 2.037 *	29.22 ±2.128 *
ALT U/L	31.58±2.2	25.6±1.910 $^{N.S}$	30.7±1.851 $^{N.S}$	28.46 ±2.649 $^{N.S}$

Table 4: concentration of AST and ALT enzymes in serum.

- Values represented by mean ± SD. N=24.
- "N.S" means no significant difference in value P≤0.05.
- "*" means a significant difference in value P≤0.05.
- Administrated groups compared to control.

Liver tissue of control group revealed normal histoarchitecture of hepatic cells and portal area (figure1A and B).The hepatocytes of rats administrated with 1.5g/Kg appeared polygonal hepatocytes connected to each other forming a network of rows of cells in normal size and shape. In the middle of cell, the nucleus is globular, dark in color and has one or two nuclei, the sinuses found in the form of wide blood vessel branching between the rows of hepatocytes, with the presence of many kupffer cells in the blood sinusoids of the liver. Figure (2-A, B). Hepatocytes in the parenchyma of the rat liver administrated with 3 g/Kg have limited enlargement, but their shape has emerged normally, small groups of hepatocytes with normal nuclei and the presence of infiltration of numbers of white blood cells around the bile ducts and surrounded by numbers of kupffer cells in the surrounding blood sinusoids. Figure (3-A, B). The hypertrophy of the cells in the parenchyma of the liver was evident in rat administrated with 6 g/Kg. It surrounding the portal area, which infiltrated large numbers of white blood cells and fibroblasts with the colloidal fibrous tissue surrounding the bile duct that had hypertrophy of epithelial cells lining the duct. The portal vein branch had congestion and presence of similar congestion in the adjacent hepatic artery. Figure(3-A) .Kupffer cells were found in the

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sinusoids, with the presence of hypertrophy of the liver cells and their appearance in the form of compact aggregates, but these cells were normal in shape, with one or two nuclei. Figure (3-B).

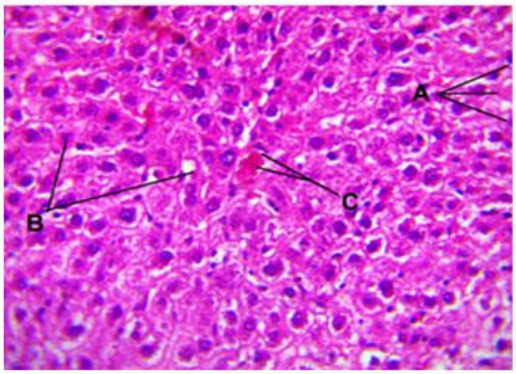

figure (1-A) section in normal rat liver, noted the rows of hepatocytes stacked with large, dark nuclei (A) central vein (B) sinusoids with kupfer cells (C) H&E X40.

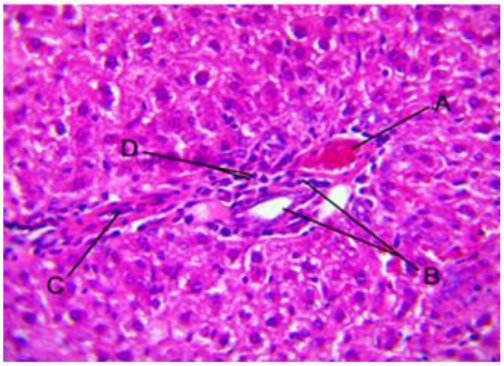

figure (1-B) section in normal rat liver, the portal area, portal vein branch filled with blood (A) biliray tract (B) hepatic artery branch (C) infiltration of white blood cells (D). H&E X40

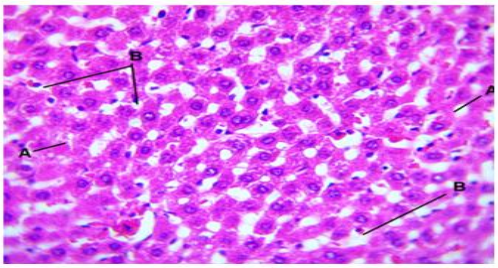

figure (2-A) section in rat liver adminsitrated with 1.5g/kg , Liver tissue, polygonal (A) hepatocytes normal shape, sinusoids are wide , branched and contained kupffer cells (B) H&E X40 .

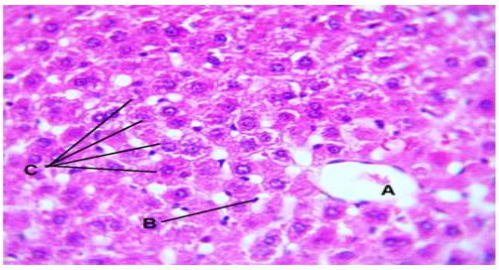

figure (2-B) section in rat liver adminsitrated with 1.5g/kg , Hepatic lobule, central vein (A). (B) kupffer cells, foamy vacuolation in the cytoplasm of (C) H&E X40.

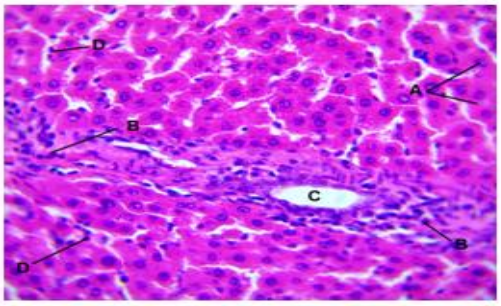

figure (3-A) section in rat liver adminsitrated with 3g/kg, Limited enlargement of hepatocytes with normal shape (A) infiltration of white blood cells (B) around the biliary tract (C) kupffer cells (D). H&E X40

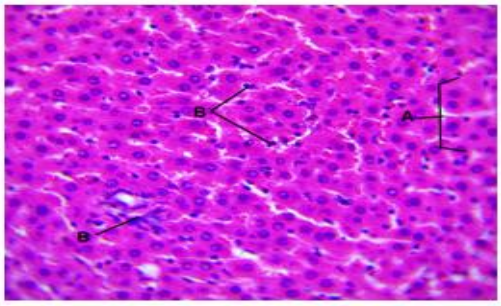

figure (3-B) section in rat liver adminsitrated with 3g/kg , hypertrophy of hepatocytes (A). (B) kupffer cells in sinusoids H&E X40 .

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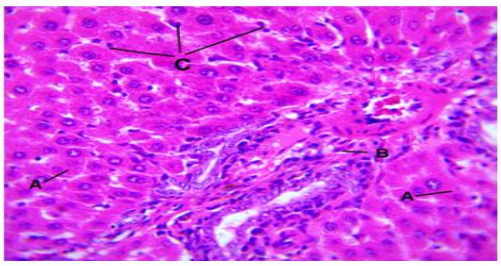

figure (4-A) section in rat liver adminsitrated with 6g/kg , Hypertrophy of hepatocytes (A) infiltration of white blood cells (B) in the portal area around the portal vein branch, the biliary tract and the hepatic artery branch, kupffer cells (C) in the sinusiods H&E X40.

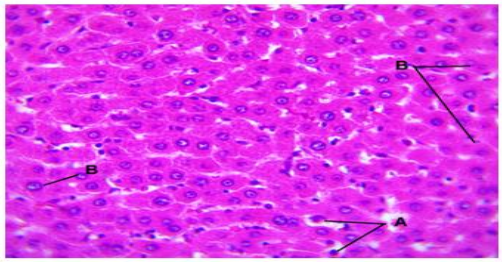

figure (4-B) section in rat liver adminsitrated with 6g/kg , kupffer cells (A) in the sinusiods, Hypertrophy of hepatocytes (B) . H&E X40.

Discussion

Rats administrated with AKP in concentrations 1.5, 3 and 6 g/ Kg contain 42.126, 84.352 and 168.704 mg/kg of amygdalin respectively (table1). Results show non- negative effect on lipid peroxidation especially in the liver (table 2), per contra it enhanced levels of glutathione and glutathione peroxidase in serum (table 1) and glutathione, Super oxide dismutase and Catalase especially in concentration 3 and 6 g/kg in homogenized liver tissue (table 2). Apricot kernel contains many bioactive materials such as tochopherol, vitamin C, saponins, oleic acid and amygdalin, there for it has antioxidant activity exhibiting anti-lipemic and increase antioxidant capacity. The study of Albogami et al. [11] indicated that amygdalin 100 mg/kg has ability to increase gene expression of glutathione peroxidase and superoxide dismutase in hepatic tissue of mice. Amygdalin hydrolysis convert it to glucose and mandelonitrile, this compound is unstable and converted to HCN and benzaldehyde [13]. Over production of benzaldehyde increase Reactive oxygen species ROS level In the cell [14]. This process stimulate and activate antioxidant system in the cell to reduce oxidative damage of ROS(table 1 and 2) Hydrocyanic acid may exert liver damage [15]. Levels of AST enzyme increased when rats treated with AKP in particular at groups administrated with 3 and 6 g/kg (table 4), While ALT was stable in compare to control (table 4). AST and ALT are the most useful indicators of liver injury. ALT is more liver specific than AST. Increasing in AST with lower ALT may refer to injury of skeletal or cardiac muscle [16-18]. Rhodanese (thiosulfate sulfurtransferase) an enzyme found in tissues of animals and plants [19]. Moreover, its distribution in tissues depends on cyanide exposure since its function is cyanide detoxification. In rats, it can be existent in liver and other tissues as mitochondrial enzyme also it is found in the brain [20]. In mitochondria, the enzyme modulate cellular respiration, thiosulfate works as sulphur donor in HCN detoxification [21] , that is may attenuate the toxicity of cyanide anion on hepatocytes and protect hepatic tissue from acute injury. Histological examination of rats administrated with 6g/kg of APK revealed a hypertrophy in hepatocytes, congestion in portal vein and infiltration of WBC, which is reflex the stress influenced by cyanide on hepatic tissue and activated antioxidant systems (table 3). Rhodanese enzyme has a protective effect against poison activity of cyanide anion [22-23-24]. Cells were in normal in shape no necrosis noticed (figure 4A-B). administration with 3 g/kg APK revealed limited enlargement of hepatocytes, infiltration of WBC, but no congestion seen in central vein or necrosis in hepatocytes (figure 3A and B) , while rats administrated with 1.5g/kg shows no alter hepatic tissue (figure 2A and B) in compare to control, so this dose consider safe.

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