A Comparative Study Between Grape (*Vitis vinifera*) Juice Varieties on Liver Toxicity Induced by Sodium Fluoride in Adult Rats

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ABSTRACT

The consumption of fruits has an important role in health protection. Grape juice is considered a healthy protecting beverage due to its high content of bioactive phenolic compounds and their antioxidant capacity. The present study was designed to compare the potential effects of three types of grape juice (Green or white-red and black) against the toxicity and tissues damage induced by sodium fluoride (NaF) in rats liver. Rats were randomly divided into five groups. The first: negative control group (6 rats) fed standard diet. From 2 to 5 group received a single oral dose 10.3 mg Naf /kg body weight for six weeks, the second group served as positive control group (6 rats), the third, fourth and fifth groups (12 rat /each) were fed standard diet and each of them was divided into two sub groups (6 rats /each) and given a daily oral dose 5 and 10 µL/g body weight for 6 weeks of the green (white), red and black grape juice respectively. After the end of the experimental period, lipids profile, liver functions, Malonaldehyde (MDA) levels, reduced glutathione (GSH Rd) and catalase (CAT) activities and histological examination of liver tissues were performed. Results showed that NaF treated rats caused elevation in lipid profile, liver functions in the serum and MDA levels with reduction in the activity of GSH Rd and CAT in liver tissues. However, treatment sodium fluoride rats with red and black grape juice reduced the levels of lipid profile, liver enzymes and MDA with enhanced activity of GSH Rd, CAT and histopathological changes in the liver tissues. While, treated rats with black grape juice (10µl/g BW) was more effective in alleviating the harmful effects of NaF in rats. In conclusion, red and black grape juice has a potent effect against NaF induced hepatotoxicity in rats and this effect might be correlated with grape antioxidant capacity.

Key words: *liver toxicity, sodium fluoride, red and black grape juice, liver functions,.*

INTRODUCTION

Fluorinated compounds such as sodium fluoride, sodium fluorosilicate and cryalite (a fluoride - containing mineral) are used in various insecticide formulations and wood preservatives **(Nabavi *et al*., 2012)**. Fluoride, an essential trace element is widely distributed in nature as its compounds or free ions. Fluorosis in human beings is mainly caused by drinking water, toothpaste, mouth rinses, burning cool, NaF dust and fumes from industries using NaF- canting salt and hydrofluoric acid, and drinking tea **(Liu *et al*., 2003)**. Fluoride easily distributes in the body through blood circulation, crosses the cellular membrane and its subsequent accumulation leads to impairment in the soft tissues **(Bouaziz *et al*., 2010)**. Excessive intake of fluoride causes adverse health effects such as fluorosis in mammals and other toxic effects on cultured tissues **(He and Chen, 2006 and GAO *et al*., 2009b)**. Also the excessive exposure to fluoride can lead to some toxicological risks as fluoride intoxication is associated with severe damage to different tissues **(Nabavi *et al*., 2012 ab).**

Chronic fluorosis may induce hyperlipidemic effect **(Khudiar and Aldabaj, 2014)** metabolic, functional and structural damages in many tissues including kidney **(Nabavi *et al*., 2013)** and liver **(Grucka - Mamczar *et al*., 2009)**. Fluoride - induced hepatotoxicity is associated with an imbalance in the oxidant antioxidant systems of hepatic tissues causes hepatic dysfunction through free radicals mediated lipid peroxidation, DNA damage, inflammation, mitochondrial dysfunction and necrotic / apoptotic cell death **(Wang *et al*., 2000 and Nabavi *et al*., 2012c)**.

The human diet, which contains many natural compounds in essential in protecting the body against the development of diseases. Human diet rich in vegetables and fruits have been associated with reduced rate of liver diseases **(Alimi *et al*., 2012)**. Grapes are one of the most valued conventional fruits in the world

(Yang and Xian, 2013) and contain various nutrient elements such as vitamins, minerals, carbohydrates, edible fibers and phytochemicals. Grape juice is a fresh and nutritional beverage, highly appreciated worldwide, which its production is significantly increasing every year **(Koyama *et al*., 2014)**. Phenolic compounds of the grape juice such as flavonoids, anthocyanins, tannins, phenolic acids, among others, are the main responsible for the beneficial healthy effects observed **(Capanoglu *et al*., 2013). Montvale (2002)** showed that red grape juice has hepatoprotective effect. Also, it is protective or therapeutic agent to attenuate organs damage and dysfunction in response to chemical toxins **(Alnahdi and Ayaz, 2012)**. Black grape can provide protection against toxic effects **(Lakshmi *et al*, 2013)**. Grape juices which are rich in polyphenol compounds with important antioxidant activity have protective effect against oxidative damage in the liver **(Rodrigues *et al*,. 2013)**.

Therefore, the present study was carried out to evaluate the protective effects of grape juice varieties on liver toxicity induced by sodium fluoride in rats.

MATERIALS &METHODS

Materials:

Grape fruit (vitis vinifera): The fresh green (white), red and black grape used in this study were purchased from the local market Shiben El-Kom City Menoufia Government, Egypt. Sodium Fluoride (Naf) was obtained from sigma Chemical Co. (St. Louis, Mo. USA). Kits for estimating biochemical analysis were purchased from Alkan Medical Company, St. El-Doky, Cairo, Egypt.

Animals: Forty eight adult male albino rats, Sprague Dawley stain, weighing 160 ± 5g were purchased from Medical Insects Research Institute, Doki, Cairo, Egypt.

Methods:

Preparation of grape juice

Fresh grape was washed with running water. Grape juice was prepared using National juicer (MJ - 176N Japan) without adding water. The pure filtrated juice was stored at -20 ºC until used.

Experimental design

Forty eight rats were housed separately in well aerated cages under hygienic laboratory conditions and fed standard diet for 7 days for adaptation according to AIN - 93 guidelines **(Reeves *et al*., 1993)**. Then rats were randomly divided into five groups; the first: negative control group (6 rats) was fed standard diet. From 2 to 5 groups received the standard diet and a single oral dose of 10.3 mg Naf /kg body weight for six weeks as described by **Blaszczyk *et al*., (2011)**, the second group served as positive control group (6 rats), the third, fourth and fifth groups (12 rats /each) on the standard diet were divided into two sub groups (6 rats /each) and given a daily oral dose 5 and 10 μL/g body weight for 6 weeks of green (white), red and black grape juice respectively. The doses of green (white), red and black grape juice was determined according to **Park *et al*., (2003)**. .At the end of the experimental period rats were anesthetized after fasting for 12h and non-heparinized blood samples were collected from the hepatic portal vein. Liver was taken and washed in saline solution until all blood was removed. The serum was collected from the blood samples by centrifugation and both serum and liver were kept frozen at - 20ºC till used for analysis.

Chemical analysis:

Total phenolics were determined according to the Folin - Ciocalteau method as described by **Kaškonienė *et al*., (2009).** Total phenolic compounds were expressed as mg gallic acid equivalents /100 ml grape juice extract. Total Flavonoid was determined using a method described by **Xu and chang (2007).** Anthocyanin was determined according to **Lako *et al*., (2007).**

Biochemical analysis:

The serum levels of total lipids, total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL.c) were determined by using methods of **Frings and Dunn (1979)**, **Allain *et al*., (1974), Fossati and Prencipe (1982)** and **Demacker *et al*., (1980)** respectively. The determination of low density lipoprotein cholesterol (LDL.c) and very low density lipoprotein cholesterol (VLDL.c) were carried out according to the methods of **lee and Nieman (1996)** as follows:

LDL.c = Total cholesterol - (HDL.c + VLDL.c).

VLDL.c = TG/5

Serum levels of alanine aninotransferase (ALT) and aspartate aminotransferase (AST) enzymes were assayed by the methods of **Moss and Henderson (1999)**. The activity of alkaline phosphatase (Alp), gamma glutamine transferase (GGT) and total bilirubin (TB) were determined by the methods of **Varley *et al*., (1980), Rosalki *et al*., (1970); Pearlman *and* lee (1974)** respectively. Malonaldehyde (MDA), reduced glutathione (GSH Rd) and catalase (CAT) were assayed according to the methods described by **Ohkawa *et al*., (1979), Moron *et al*., (1979)** and **sinha (1972)** respectively.

Histopathology examinations:

Small specimens of the organs liver were taken from each experimental group, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in zylene and embedded in paraffin. Sections of 4–6 µm thickness were prepared and stained with hematoxylin and eosin according to **Bancroft *et al*., (1996).**

Statistical analysis:

Results were expressed as the mean ± SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program **(Artimage and Berry, 1987)**.

RESULTS & DISCUSSION

Total phenolics, total flavonoids and anthocyanins of fresh green (white), red and black grape juice is presented in Table (1). Black grape juice had the highest ($P \leq 0.05$) total phenolic, total flavonoids and anthocyanin contents followed by red grape juice, while green (white) grape juice was lowest (P≤0.05) in its content of total phenolic, total flavonoids and anthocyanin.. Grapes contain high amounts of phenolic, flavonoids and anthocyanins and acts as antioxidant **(Yildirim *et al*., 2005). Liang *et al*., (2014)** reported that the content of Vitis vinifera grape cultivars of total phenolics ranged from 95.3 to 686.5 mg/100g and flavonoids from 94.7 to 1055 mg/100g .Also **Mitic *et al*., (2011)** showed that red fruit juices contain a high content of a different group of polyphenols, which have a potent antioxidant capacity and found that black grape juice had total phenolic (2230.4 mg gallic acid equivalent (GAE)/L) total flavonoids (368.48 mg catechin equivalent (CE)/L) and anthocyanins (208.67 mg cyaniding-3-glucosides equivalent (C3GE)/L). **Toaldo *et al*., (2015)** found that white grape juice had lower total phenolic content compared with red grape juice, whereas red grape juice was higher in anthocyanins content than white grape juice. The content of total phenolic of the grape juices was 1151 mg GAE/L reported by **Ishimoto *et al*., (2006)**. **Kulcan *et al*., (2015**) showed that total anthocyanin of extracted raw grape juice was 48.46 mg/L. Moreover, total anthocyanin content varied from 181.2 to 716.4 mg/100g fresh weight in grape varieties **(Nile *et al*., 2015).**

Data in Table (2) shows effect of green (white), red and black grape juice on serum lipid profile of hepatotoxicated rats. The results indicated that the levels of total lipids, cholesterol, triglyceride, VLDL.c and LDL.c showed significant (P≤0.05) increase, while the level of HDL.c significantly (P≤0.05) decreased in the sodium fluoride groups compared to negative control group. Similar results were obtained by **Abdel-Wahab (2013)** reported that oral administration of NaF induced a significant increase in the level of total lipids, triglycerides and total cholesterol. Also, **Hassan and Yousef (2009)** found that the treatment with NaF caused significant increase in plasma levels of total lipid, total cholesterol, triglyceride and LDL.c and decrease in HDL.c. The obtained results in the present study may be attributed to high levels of NaF lead to its accumulation in the liver leading to disturbance of lipid metabolism and in turn to the reported elevation the lipid profile **(Grucka - Mamczar *et al*., 2004)**.

Sodium fluoride intoxicated rats treated with green (white), red and black grape juice had significant reduction (P≤0.05) in cholesterol, triglyceride, VLDL.c and LDL.c levels compared to positive control group. **Shanmuganayagam *et al.*, (2007)** reported that the daily consumption of grape juice at 70 ml/kg/day decreased TC by 24% in rabbits. Administration of 10 µl/g BW of green (white), 5,10 µl/g BW of red and black grape juice caused a significant reduction (P≤0.05) in total lipids level in sodium fluoride intoxicated rats, while HDL.c had an opposite trend. On the other hand, the levels of total lipids and LDL.c were lower (P≤0.05) in sodium fluoride intoxicated rats treated with red and black grape juice than that of rats treated with green (white) grape juice. The study of **Castilla *et al*., (2006)** on healthy volunteers reported that concentrated red grape juice decreased LDL.c and increased HDL.c as well as in hemodialysis patients, polyphenol from red grape might lead to a possible modifying effect of lipoprotein metabolism through hepatic removal of cholesterol and an increase in its fecal excretion. Also, the present study showed that cholesterol, triglyceride and VLDL.c levels were significantly decreased (P≤0.05) in sodium fluoride intoxicated rats treated with 10 µl/g BW of red and 5,10 µl/g BW of black grape juice compared with rats treated with green (white) grape juice. **Vinson *et al*., (2001)** found that grape juice decreased both TC and LDL.c in hamesters. However, treatments with 10 µl/g BW of black grape juice was more effective (P≤0.05) in reducing total lipids, cholesterol, triglyceride, VLDL.c and LDL.c levels in sodium fluoride intoxicated rats than those treated with 10 µl/g BW of green (white) grape juice, 5,10 µl/g BW of red grape juice and 5 µl/g BW of black grape juice. Moreover, there were no significant differences (P>0.05) in cholesterol, triglyceride, VLDL.c and LDL.c levels between black grape juice (10 µl/g BW) and negative control group. These results may due to high level of polyphenols (total phenolic, flavonoids and anthocyanin) present in grape juices. The action of polyphenols is associated with modulation of important physiological parameters such as plasma lipid profile, as a result of improved resistance towards oxidative stress, inflammation and endothelial dysfunction reported by **Van Duynhoven *et al*., (2010).** Also, **Alnahdi and Ayaz (2012)** reported that phytochemical constituents of the grape juice have hypolipidemic potential action.

Effect of green (white), red and black grape juice on liver functions of hepatotoxicity rats is illustrated in Table (3). From the table it can be observed that treated rats with sodium fluoride caused significant increased (P≤0.05) in the activities of AST, ALT, ALP, GGT and TB. These results agreed with the findings of **Abdel -Wahab (2013)** who found that exposure to NaF (10 mg/kg/day) for 4 weeks resulted in impairment in liver functions through significant increase in the activity of AST, ALT, ALP and total bilirubin by 73.1%, 131.8%, 63.2% and 310.4% respectively. Also, **Shanthakumari *et al*.**, (2004) recorded a significant increase in plasma ALT, AST and ALP of rats treated with 25 ppm of fluoride for 8 and 16 weeks. The increased activities of serum AST, ALT and ALP indicate that the liver is susceptible to NaF induced toxicity. This increase could be attributed to hepatic damage resulting either in increased release of functional enzymes from biomembranes, or the increased synthesis as reported by **Muthumani and Milton Prabu (2012)**. The elevation in the concentration of serum bilirubin in NaF - treated rats is consistent with the presence of hepatic damage **(Nabavi *et al*., 2012c)**.

On the other hand, this study showed significant reduction (P≤0.05) of AST, ALT, GGT,ALP and TB in sodium fluoride intoxicated rats after treating them with green(white) (10µl/g BW), red and black grape juices (5, 10 µl/g BW). These results are in accordance with **Pirinccioglu *et al*., (2012)** who reported that Okuzgozu grape juice significantly reduced the elevated activities of AST, ALT, ALP and TB and the improved the functional status of the liver. Administration of red grape juice (2ml/rat) daily for 4 week by **Alnahdi and Ayaz (2012)** ameliorated the alteration in ALT and AST. However, in this study treated sodium fluoride intoxicated rats with black grape juice (10 µl/g BW) showed (p≤0.05) low values of AST, ALT, GGT, ALP and TB compared to black grape juice (5µl/g BW) and red grape juice (5,10 µl/g BW). . The procyanidins found in grapes can inhibit the apoptosis and damage of cells by oxygen free radicals **(Li and Zhong, 2004)**. Therefore, the potent effect of grape juice may be the potent antioxidant effect of its polyphenols, including phenolic acids, anthocyanins and flavonoids (eg. proanthocyanidins), whereas phenolic compounds and flavonoids possess hepatoprotective activity in various experimental models as reported by **Monagas *et al*, (2003) and sharma *et al*., (2012)**.

The results in Table (4) show effect of green (white), red and black grape juice on MDA, GSH.Rd and catalase activity in liver homogenates of hepatotoxicated rats. A significant elevation in the level of MDA and reduction in the activities of GSH.Rd and catalase in the liver were observed in sodium fluoride intoxicated rats when compared with negative control group (p≤0.05). Similar results were obtained by **Abdel-Wahab (2013)** and **Nabavi *et al*, (2013)** who reported that NaF intoxication resulted in a significant increase in lipid peroxidation as evidenced by the increased MDA level, whereas the activities of reduced glutathione (GSH.Rd) and catalase (CAT) were reduced in hepatic tissues. Naf is known to produce oxidative damage in the liver by enhancing peroxidation of membrane lipids, a deleterious process solely carried out by free radicals **(Pieta *et al.*, 2012)**. Impairment of the antioxidant defense system is considered to be critically involved in NaF-induced toxic effects. This impairment interferes with the elimination of lipid peroxidation products and causes their accumulation in the cells leading to the damage of cell membranes reported by **Abdel-Wahab (2013)**.

On the other hand, sodium fluoride intoxicated rats treated with red and black grape juice (5,10 µl/g BW) had lower MDA in the liver than those rats treated with green(white) grape juice (10 µl/g BW) ($P<0.05$). Also, treatment with 10 µl/g BW of black grape juice was more effective in reducing MDA by 47,691% compared with positive control group ($P<0.05$) . No Significant differences ($P>0.05$) was found in the levels of MDA among sodium fluoride intoxicated rats treated with 10 µl/g BW of black grape juice and negative control group. The present results were in the same trend with **Toaldo *et al.*, (2015)** who found that grape juice ingestion promoted a significant decrease in thiobarbituric acid reactive substances (TBARS) levels compared to the control intervention, demonstrating the protective effect of juice consumption against lipid peroxidation. Also, the reduction in MDA levels after treating Okuzgozu grape juice may be due to its high content of flavonoids and anthocyanin **(Pirinccioglu *et al.*, 2012)** Furthermore, the activities of GSH Rd and CAT were significantly increased ($P\leq0.05$) in livers of sodium fluoride intoxicated rats treated with green (white) grape juice (10 µl/g BW), red and black grape juice (5, 10 µl/g BW) as compared with positive control group. However, sodium fluoride intoxicated rats treated with 10µl/g BW of black grape juice had high activity of GSH Rd and CAT compared to other concentrations of different types grape juice ($P\leq0.05$). **Lakshmi *et al.*, (2013)** reported that black grape extract showed significant increase in GSH Rd and CAT activities as well as decrease in MDA levels in rat liver compared with lead control group. Treatment with organic and conventional purple grape juices conferred protection against lipid and protein oxidative damage through limited increase in TBARS levels and inhibited reduction of catalase activity in the liver (**Rodrigues *et al*., 2013)**.. All of these results are in accordance with the content of bioactive polyphenol compounds in grape juice, which could play a role against lipid peroxidation. **Gris *et al*., (2013)** showed that the improvement of the anti-oxidative defense was promoted by grape juice ingestion due to the capacity of phenolic compounds that eliminate free radicals and prevent lipid peroxidation by scavenging peroxyl radicals in phospholipids membrane of the cells. Black grape juice was capable of reducing carbonyl and lipid peroxidation levels in the liver and induced better antioxidant effects because of its content of anthocyanin **(Dani *et al.*, 2008)**.

Photo (1) shows effect of green (white), red and black grape juices on histological examination of liver tissues in hepatotoxicity rats. Histopathological examination of the liver of normal control rats revealed normal histological structure without any pathological lesions (H&EX 400) (Photo1A). While the examination of sodium fluoride intoxicated rats liver tissues showed congestion of the control veins and hepatic blood vessels with sinusoidal dilatation. The parenchymal hepatocytes showed various degenerative changes mostly centrilobular including granular and vacuolar degeneration with activated kupffer cells and necrosis of the hepatocytes without any nuclear structure (Photo 1B). Histological sections of livers in Naf treated rats revealed hepatic injury manifested by mononuclear cell aggregation around the congestive blood vessel and bile duct in the buccal area together with dilatation of the sinusoid reported by **Khudiar and Aldabaj (2015)**.

Also, **Atmaca *et al*., (2014)** showed that fluoride intoxication was associated with severe histopathological changes in liver tissues.

As shown in (Photo 1C) portal area in liver of sodium fluoride intoxicated rats treated with 5µl/g BW of green (white) grape juice showed mild fibroplasia, mild bile duct hyperplasia and inflammatory cells infiltration. The changes in rats treated with 5µl/g BW of red grape juice were focal area of necrotic hepatocytes replaced by mononuclear inflammatory cells (Photo 1D). However, only sinusoidal dilatation and mild hepatocellular degeneration were observed in liver of sodium fluoride intoxicated rats treated with 5µl/g BW of black grape juice (Photo 1E). On the other hand, fluoride rats treated with 10 µl/g BW of green (white) grape juice revealed mild hyperplasia of the bile duct with few inflammatory cells infiltration (Photo 1F).. Moreover, the liver of sodium fluoride rats treated with 10µl/g BW of red and black grape juice showed mild kupffer cell activation and few necrotic cells but with normal organization of the hepatic cords (Photo 1G and H). So the histological examination of liver tissues of sodium fluoride intoxicated rats treated with 10µl/g BW of green (white), red and black grape juices had nearly the same moderate degree of restorative effect on the hepatic structure against the harmful effect of NaF. **Pirinccioglu *et al*., (2012)** found that administration of grape juice resulted in the restoration of the pathology of the liver tissue to some extent. Grape juices present important hepatic and systemic protection effects against oxidative damages in rats **(Rodrigues *et al*, 2013).** Quercetin (the most abundant flavonoids in black grape juice) was reported as a protective agent against oxidative damage in rat hepatocytes reported by **Liu *et al*., (2009),** Whereas flavonoids effectively prevent lipid peroxidation and protein oxidation in rats liver mitochondria **(Londhe *et al*., 2009)**.

CONCLUSION

These results have suggested that red and black grape juice contain a high content of different group of polyphenols, which have a potent antioxidant capacity and potent effects against the toxicity of NaF through inhibition of the development of fluoride - induced hepatotoxicity in rats. Accordingly, care must be taken into account to ovoid mammalian and human exposure to NaF and attention should be paid to sources of it in foods and water as well as occupational sources.

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Table (1): Total phenolic, total flavonoids and anthocyanins of fresh green (white), red and black grape juice.

Parameters	Green (white) grape juice	Red grape juice	Black grape juice
Total phenolic (mg gallic acid/100ml)	$53.41^{c} \pm 2.81$	$125.6^{b} \pm 2.8$	$172.8^{a} \pm 3.01$
Total Flavonoids (mg catechin/100 ml)	$14.75^{c} \pm 0.75$	$22.74^{b} \pm 1.87$	$32.42^{a} \pm 3.49$
Anthocyanins (mg cyanidin-3-glucoside/100ml)	$0.89^{c} \pm 0.34$	$72.47^{b} \pm 2.65$	$95.34^{a} \pm 0.79$

Each value in the table is the mean ± standard deviation of three replicates.

Table (2): Effect of green (white), red and black grape juice on serum lipid profile of hepatotoxicity rats

Groups / Parameters	Negative control	Sodium Fluoride groups						
		Positive control	**Green (white) grape juice**		**Red grape juice**		Black grape juice	
			(5µl/g BW)	**(10 µl/g BW)**	**(5µl/g BW)**	**(10 µl/g BW)**	**(5µl/g BW)**	(10 µl/g BW)
Total lipids (mg/dl)	421.96 ^{f} ± 11.03	604.71 ^{a} ± 10.24	595.5 ^{a} ± 14.49	569.67 ^{b} ± 9.42	540.5 ^{c} ± 9.65	502.08 ^{d} ± 10.12	493.5 ^{d} ± 8.67	**440.17 ^{e} ± 9.54**
Cholesterol (mg/dl)	97.38 ^{f} ± 1.34	170.33 ^{a} ± 7.5	162.67 ^{b} ± 6.15	140.83 ^{c} ± 9.02	134.17 ^{c} ± 7.63	109.33 ^{e} ± 5.47	117.67 ^{d} ± 5.2	**101.33 ^{f} ± 6.02**
Triglyceride (mg/dl)	66.86 ^{f} ± 2.18	114.03 ^{a} ±3.96	104.42 ^{b} ± 4.91	93.47 ^{c} ± 3.66	91.95 ^{c} ± 5.32	77.33 ^{e} ± 3.78	84.46 ^{d} ± 2.24	**70.21 ^{f} ± 1.84**
HDL.c (mg/dl)	56.97 ^{a} ± 1.67	13.75 ^{g} ± 1.33	20.92 ^{f} ± 2.87	26.25 ^{e} ± 4.19	28.17 ^{e} ± 4.49	42.38 ^{c} ± 4.47	36.39 ^{d} ± 3.94	**52.38 ^{b} ± 4.47**
VLDL.c (mg/dl)	13.37 ^{f} ± 0.44	22.81 ^{a} ± 0.79	20.88 ^{b} ± 0.98	18.69 ^{c} ± 0.73	18.39 ^{c} ± 1.06	15.47 ^{e} ± 0.76	16.89 ^{d} ± 0.45	**14.04 ^{f} ± 0.36**
LDL.c (mg/dl)	**27.04 ^{g} ± 0.77**	**133.77 ^{a} ± 7.79**	**120.87 ^{b} ± 7.25**	**95.89 ^{c} ± 7.79**	**87.61 ^{d} ± 7.77**	**51.48 ^{f} ± 6.16**	**64.39 ^{e} ± 4.93**	**34.91 ^{g} ± 8.31**

Values in the table were expressed as means ± SD. Different letters in the same now were significantly different ($p \leq 0.05$).
HDL.c : high density lipoprotein cholesterol, VLDL.c : very low density lipoprotein cholesterol, LDL.c : low density lipoprotein cholesterol.

Table (3): Effect of green (white), red and black grape juice on liver function of hepatotoxicity rats.

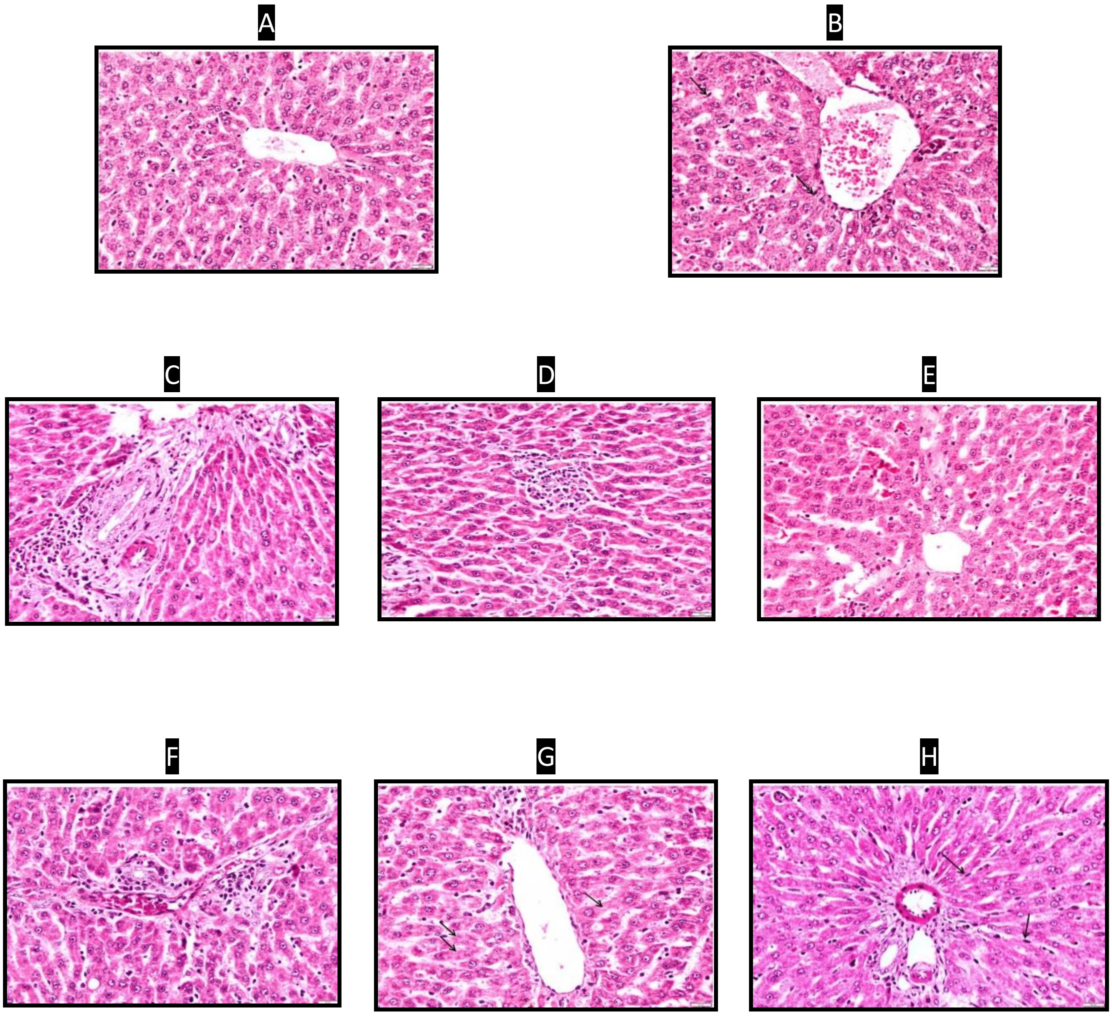
Groups / Parameters	Negative control	Sodium Fluoride groups						
		Positive control	**Green (white) grape juice**		**Red grape juice**		Black grape juice	
			(5µl/g BW)	**(10 µl/g BW)**	**(5µl/g BW)**	**(10 µl/g BW)**	**(5µl/g BW)**	(10 µl/g BW)
AST (U/I)	70.83 ^{g} ± 2.4	125.33 ^{a} ± 12.08	120.33^{a} ± 2.8	109.5 ^{b} ± 2.43	103 ^{c} ± 2.61	93.83 ^{d} ± 3.06	86.17 ^{e} ± 3.19	**77.5 ^{f} ± 1.87**
ALT (U/I)	30.09 ^{f} ± 2.35	67.5 ^{a} ± 3.62	56.5 ^{b} ± 3.08	55.17 ^{b} ± 3.54	48.5 ^{c} ± 4.28	42.62 ^{d} ± 2.42	41.5 ^{d} ± 2.43	**36.17 ^{e} ± 3.76**
GGT (U/I)	33.67 ^{f} ± 2.88	80.02 ^{a} ±2.64	78.33 ^{a} ± 3.14	72.11 ^{b} ± 2.54	63.67 ^{c} ± 2.16	51.17 ^{d} ± 2.93	53.5 ^{d} ± 3.83	**40.31 ^{e} ± 3.48**
ALP (U/I)	116.13 ^{g} ± 2.58	182.88 ^{a} ± 2.91	178.83 ^{a} ± 3.19	166.33 ^{b} ± 5.65	161.5 ^{c} ± 4.09	150.17 ^{d} ± 3.97	142.88 ^{e} ± 3.4	**128.44 ^{f} ± 2.02**
TB (mg/dl)	**0.42 ^{d} ± 0.1**	**0.99 ^{a} ± 0.3**	**0.93 ^{a} ± 0.36**	**0.85 ^{ab} ± 0.04**	**0.74 ^{bc} ± 0.31**	**0.69 ^{c} ± 0.02**	**0.62 ^{c} ± 0.03**	**0.48 ^{d} ± 0.04**

Values in the table were expressed as means ± SD. Different letters in the same now were significantly different ($p \leq 0.05$).
AST: aspartate aminotransferase, ALT : alanine aminotransferase, ALP : alkaline phosphatase, GGT : gamma glutamine transferase, TB : total bilirubin.

Table (4): Effect of green (white), red and black grape juice on MDA, GSH and Catalase activity in liver homogenate of hepatotoxicity rats

Groups / Parameters	Negative control	Sodium Fluoride groups						
		Positive control	Green (white) grape juice		Red grape juice		Black grape juice	
			(5µl/g BW)	(10 µl/g BW)	(5µl/g BW)	(10 µl/g BW)	(5µl/gBW)	(10 µl/g BW)
MDA (Mmol / g.tit)	27.16 ^{e} ± 8.93	61.14 ^{a} ± 3.44	59.24 ^{a} ± 2.71	54.19 ^{b} ± 3.04	49.26 ^{c} ± 3.38	43.26 ^{d} ± 3.71	39.72 ^{d} ± 1.57	**31.98 ^{e} ± 2.11**
GSH Rd (Mg / g.tit)	17.45 ^{a} ± 1.49	7.86 ^{f} ± 1.28	8.01 ^{f} ± 0.64	9.49 ^{e} ± 1.31	11.02 ^{d} ± 0.85	12.55 ^{c} ± 1.14	12.28 ^{cd} ± 1.48	**14.95 ^{b} ± 1.15**
CAT (Mmol/ g.tit)	**80.7 ^{a} ± 1.8**	**47.2 ^{f} ± 3.04**	**49.35 ^{f} ± 1.91**	**52.09 ^{e} ± 2.78**	**54.73 ^{e} ± 1.8**	**61.09 ^{d} ± 1.58**	**65.64 ^{c} ± 2.1**	**75.09 ^{b} ± 3.03**

Values in the table were expressed as means ± SD. Different letters in the same Raw were significantly different ($p \leq 0.05$).
MDA: malonaldehyde, GSH.Rd : reduced glutathione, CAT: catalase.



A: negative control, B: positive control (sodium fluoride group untreated), c: sodium fluoride group treated with 5µl/g BW of green (white) grape juice, D: sodium fluoride group treated with 5µl/g BW of red grape juice, E: sodium fluoride group treated with 5µl/g BW of black grape juice, F: sodium fluoride group treated with 10 µl/g BW of green (white) grape juice, G: sodium fluoride group treated with 10µl/g BW of red grape juice H: sodium fluoride group treated with 10µl/g BW of black grape juice.

Photo (1): Effect of green (white), red and black grape juice on histological examination of liver tissues in hepatotoxicity rats.

دراسة مقارنة بين أنواع مختلفة من عصير العنب على تسمم الكبد الناجم عن فلوريد الصوديوم فى الجرذان البالغة

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استهلاك الفواكه يلعب دورا هاما كعامل حماية للصحة ، ويعتبر عصير العنب مشروب وقائى صحى نظرا لمحتواه العالى من المركبات الفينوليه الفعالة ونشاطها المضاد للأكسدة . لذا فقد صممت الدراسة الحالية لمقارنة التأثيرات المحتملة لثلاثة أنواع من عصير العنب [الأخضر(الأبيض) ، الأحمر و الأسود] ضد تسمم وتلف الأنسجة الناجم عن فلوريد الصوديوم فى كبد الجرذان. تم تقسيم الجرذان إلى خمس مجموعات ، المجموعة الأولى كانت بمثابة المجموعه الضابطة السالبة (٦ جرذان) تغذت على الوجبة الضابطة ، المجموعات من الثانية للخامسة تتناول جرعة واحدة عن طريق الفم ١٠,٣ ملجم/كجم من وزن الجسم من فلوريد الصوديوم لمدة ستة أسابيع والمجموعة الثانية كانت بمثابة المجموعة الضابطة الموجبة (٦ جرذان) ، المجموعة الثالثة ، الرابعة والخامسة (١٢ جرذ بكل منها) تغذت على الوجبة الضابطة وتم تقسيم كل مجموعة منها إلى مجموعتين فرعيتين (٦ جرذان بكل منها) وأعطيت جرعة يومية عن طريق الفم ٥ ، ١٠ ميكروليتر/جرام من وزن الجسم لمدة ٦ أسابيع من عصير العنب الأخضر(الأبيض) ، الأحمر والأسود على التوالى. بعد انتهاء فترة التجربة ، تم تقييم صورة دهون الدم ، وظائف الكبد ، مستوى المالوندالدهيد (MDA) ، نشاط الجلوتاثيون المختزل (GSH.Rd) والكتاليز(CAT) والفحص النسيجى لأنسجة الكبد. وقد أظهرت النتائج أن معاملة الجرذان بفلوريد الصوديوم أدى إلى ارتفاع دهون الدم ، وظائف الكبد فى السيرم ومستويات المالوندالدهيد(MDA) مع انخفاض نشاط الجلوتاثيون المختزل (GSH.Rd) والكتاليز(CAT) فى أنسجة الكبد ، فى حين أن معاملة الفئران المصابة بالتسمم بفلوريد الصوديوم بعصير العنب الأحمر والأسود قد خفضت من مستويات دهون الدم ، انزيمات الكبد و المالوندالدهيد (MDA) مع تحسن نشاط الجلوتاثيون المختزل (GSH.Rd) والكتاليز(CAT) والتغيرات الهستوباثولوجية فى أنسجة الكبد ، بينما معاملة الجرذان بعصير العنب الأسود بجرعه (١٠ ميكروليتر/ جرام من وزن الجسم) كانت أكثر فاعلية فى التخفيف من الآثار الضارة لفلوريد الصوديوم فى الفئران. وقد خلصت الدراسة إلى أن عصير العنب الأحمر والأسود له تأثير فعال ضد تسمم الكبد الناجم عن فلوريد الصوديوم فى الجرذان وأن هذا التأثير قد يرتبط بنشاط عصير العنب المضاد للأكسدة.

الكلمات الكشافة : *تسمم الكبد – فلوريد الصوديوم – عصير العنب الأحمر والأسود – وظائف الكبد.*