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Efficacy Of Bitter Apple Fruit (Citrullus Colocynthis) Consumption In Rats With Induced Steatohepatitis

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Abstract

The study was conducted to investigate the effect of bitter apple fruit (citrullus colocynthis) consumption in rats with induced steatohepatitis. Forty adult male albino rats, weighing (200 ± 10 g) were divided into 2 main groups, the first main group (n=8 rats) was fed on basal diet during the experimental period and kept as a negative control group. The second main group (n=32 rat) was fed on basal diet with methionine- choline deficient diet (MCDD) to induce steatohepatitis, then were divided into four subgroups as follow: Subgroup (1) positive control was fed on a MCDD only. Subgroups (2-4) were fed on a MCDD supplemented with dried bitter apple at 5, 7 and 10% respectively for 8 weeks. The results indicated that diet supplemented with bitter apple at different levels significantly (P< 0.05) increased the final body weight, body weight gain % and FER compared to the positive control group. Moreover, significant decrease (P< 0.05) in the mean level of MDA, and significant increase (P< 0.05) in the mean level of CAT was observed due to bitter apple supplementation at different levels caused. In addition to an improvement of serum liver enzymes and serum of lipid profile due to bitter apple supplementation, while serum HDL-C was significantly increased (P< 0.05) as compared to the control positive group. So that, it could be concluded that, bitter apple may be given to patients suffer from liver diseases.

Key Words: bitter apple – steatohepatitis – methionine – choline – inflammatory – antioxidant - Rats.

Introduction

Fatty liver indicates a large range of diseases described by excessive fat deposit in the liver, which could be alcoholic or non-alcoholic in origin. With common pathological changes in the liver that range from simple nonprogressive steatosis to non-alcoholic steatohepatitis (NASH) **(Brunt, 2004 and Soderberg et al., 2010).** NASH is associated with visceral obesity, insulin resistance, dyslipidemia, and type II diabetes mellitus (**Yamaguchi et al., 2007 and Cusi, 2009**).

Alcoholic liver disease (ALD) is one of the most reasons of liver injury, reporting for 3.8% of all global deaths and 4.6% of global disability-adjusted life-years attributable to alcohol **(Rehm et al., 2009).** Excessive alcohol drinking has long been known as an important risk factor for the growth of liver disease **(Yip and Burt, 2006). M**any pathways have been submitted in which oxidative stress plays a key role in alcohol-induced liver injury **(Cederbaum, 2001 and Arteel, 2003)**

Liver is responsible for metabolizing alcohol in the body, by alcohol dehydrogenase, aldehyde dehydrogenase and cytochrome **(Agarwal, 2001**). It is particularly relevant to the development of ALD

caused by the generation of alcohol-induced reactive oxygen species (ROS) **(Wang, 2010),** which leads to lipid peroxidation in liver cells, and changes of alcohol metabolizing enzymes (**Charles et al., 1985).** In addition, alcohol consumption caused the production of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), which causes hepatocellular damage **(Petrasek et al., 2011).**

Interest concerned about the use of alternative medicines for the treatment of hepatic disease has been developed. Bitter apple (citrullus colocynthis), is a strong nutrient-dense plant consists of many compounds including bioactive chemicals, vitamins and minerals that provide significant usefulness in treating a wide range of illnesses **(Bakare et al., 2010)** as well as their contents in dietary fiber and high antioxidant compounds (phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates) **(Snee et al., 2011).**

Bitter apple exhibits numerous biological impacts, such as regulation of glucose metabolism and blood lipids, **(Tsai et al., 2012)** in addition to its antioxidant, **(Thenmozhi et al., 2011),** anti-inflammation **(Hsu et al., 2012),** anti-tumour **(Nerurkar and Ray, 2010)** and antimicrobial activities **(Omoregbe et al., 1996).**

Materials and methods

Materials: Dried Bitter apple was purchased from local market. Casein, vitamins, minerals, DL-methionine, starch and cellulose were obtained from Morgan Company for Chemicals, Cairo, Egypt. Sucrose and oil were bought from the local market. **Kits** for blood analysis was obtained from Gama Trade Company for Chemicals. Forty adult male albino rats weighing (200 ± 10 gm) were obtained from Helwan Farm, Ministry of Health and Population.

Methods: The scientific identification of Bitter apple was carried out at the Agriculture Research Centre, that include, **Kingdom**: Plantae, **Subkingdom**: Tracheobionta, **Super division**: Spermatophyta, **Division**: Magnoliophyta, **Class**: Magnoliopsida, **Subclass**: Dilleniidae, **Order**: Cucurbitales, **Family**: Cucurbitaceae , **Genus** :Citrullus, **Species epithet**: Colocynthis.

Chemical composition: Dried Bitter apple were analyzed by the standard methods for moisture, protein fat, ash and crude fiber according to **A.O.A.C, (2012)**. Total carbohydrate was calculated by difference. **Minerals and vitamins** including calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), sodium (Na) and zinc (Zn) were determined according to the method of **A.O.A.C, (2012)** using Atomic Absorption Spectrophotometer, Perkin-Elmer Model 2380 manufacture, USA, also vitamin B complex was determined according to **Hossain et al., (2010).**

Total antioxidant capacity (TAC), total phenolic content (TPC) and total flavonoid content (TFC) of the samples were determined by the method of **(Prieto et al., 1999; Singleton et al., 1999 and Sarikurkcu et al., 2009).** The saponin, Phytate, alkaloids and tannins content were determined following the methods of (**Obadoni and Ochuko, 2001; Harborne, 1973; Abaza et al., 1968 and Makkar et al., 1993),** respectively.

The biological experiment and biochemical analyzes were conducted at the graduate lab of Faculty of Home Economics, Helwan University. The basal diet was consisted of 100 g sucrose (g/kg

diet), 200 g casein (> 80 % protein), 560.7 g corn starch, 40 g corn oil, 50 g cellulose, 35 g mineral mixture, 10 g vitamin mixture, 1.8 g L-Cystine, and 2.5 g choline bitartrate **(Reeves et al., 1993).**

Forty adult male albino rats, weighing (200 ± 10 g) were housed in well-aerated wire cages. All animals were kept under normal healthy condition and were fed on basal diet for one week for adaptation. Rats were divided into two main groups, The first main group (n=8 rats), kept as negative control and was fed on basal diet during all the experimental periods. The second main group (n=32 rats) was fed on basal diet with methionine- choline deficient diet (MCDD) to induce steatohepatitis **(Veteläinen et al., 2007).** To ensure the occurrence of steatohepatitis, eight rats from the 2nd main group was sacrificed after one and five weeks of feeding the MCDD, then liver was obtained for histopathological examination. Hepatocytes screening was showed that <30% the cells were affected without inflammation, while severe steatosis was reached after 5 weeks (> 60% of the hepatocytes were affected with inflammation) **(Veteläinen et al., 2007).**

The rest of rats (n=24 rats) with induced steatohepatitis were fed on MCDD then were divided into four subgroups as follow: Subgroup (1): was served as positive control and was fed on a MCDD only to the end of the experiment, while the other subgroups from (2-4) were fed on a MCDD supplemented with 5, 7 and 10 % of dried bitter apple, respectively.

All rats were observed each day. Their feed intake (FI) was determined daily, and body weights were obtained every week. Feed Efficiency Ratio (FER) and body weight gain percent (BWG%) was calculated according to **(Champman et al., 1955)** using the following equation:

$$\textbf{Body weight gain } \% = \frac{\textbf{Final body weight} - \textbf{Initial body weight}}{\textbf{Initial body weigh}} \times \mathbf{100}$$

$$\mathbf{FER} = \frac{\text{Body weight gain (g/d)}}{\text{Feed intake (g/d)}}$$

At the end of experimental period (8 weeks), rats were sacrificed after overnight fasting and blood of each rat was taken from the abdominal aorta under anesthesia by diethyl ether. The serum was separated by leaving the blood samples 15 minutes at room temperature then were centrifuged at 3000 rpm for 20 minutes, then was kept in plastic vials at -20°C until biochemical analysis**.**

Biochemical Analysis: Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to **Bergmeyer et al., (1978),** and alkaline phosphatase (ALP) was determined according to **Belfield and Goldberg (1971).** Serum total cholesterol (TC) **(Richmond, 1973),** triglycerides (TG) **(Wahlefeld, 1974),** high density lipoprotein (HDL-c) **(Albers et al., 1983),** were analyzed according to the reported methods. Meanwhile, low density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c) were calculated according to (**Fridewald et al., 1972)** using the following equation: LDL-c = TC - (HDL-c + VLDL-c), VLDL-c = TG/5, respectively. Serum Malondialdehyde (MDA) were measured using the thiobarbituric acid reaction method of **Placer et al., (1966). Serum** Catalase activity was measured following the method of **(Aebi, 1984)**.

Statistical analysis: The obtained data was statistically analyzed using the Statistical Package for Social Science (SPSS), Version 23.0**.** One way analysis of variance (ANOVA) was used for comparing among groups. P value of less than 0.05 was considered to indicate statistical significance **(Snedecor and Cochran, 1980).**

Results

Table (1): chemical composition of dried bitter apple:

Nutrient composition (g/100g)	
Moisture	4.91
Ash	2.00
Protein	13.19
Fat	18.59
Linoleic acid (68.69%)	
Oleic acid (12.91%)	
Palmitic acid (9.33%)	
Stearic acid (6.75%)	
Mineral composition (g/100g)	
Calcium	560
Iron	10
Magnesium	205
Phosphorus	32
Sodium	11
Zinc	1.5
Vitamin B complex (mg/100 g)	
Thiamine	1.4
Niacin	0.24
Pyridoxin	2.2
Folate	0.5

Data in Table 1 demonstrated that bitter apple had high amount of fats, including high percent of unsaturated fatty acids as palmitic, stearic, oleic, and linoleic. The percent of linoleic acid was the most abundant fatty acid, constituting 68.69 % of the total fatty acids, while Stearic acid was the lowest percentage 6.75%. Bitter apple also contains a high percent of protein, minerals as well as vitamin B complex. Dried bitter apple provide high percentage of Calcium, Magnesium while provide low level of Na

Table (2): Phytochemicals content in dried bitter apple

Parameters		
TAC (µg ml^{-1})	DPPH method	1.42
	ABTS method	711.22
TPC (mg GAE/g)		21.40
TFC (mg QE/g)		55
Saponin (mg)		21.34
Phytate (mg)		0.80
Alkaloids(mg)		3.20
Tannins(mg)		20

TAC=total antioxidant content , TPC= total phenolic content , TFC= total flavonoid.

Table (2) showed the phytochemicals content in dried bitter apple. From this table it was observed that dried bitter apple is rich in total antioxidant capacity, Total phenolic and flavonoid content. While, dried bitter apple contains low amount of phytate, saponin, alkaloids and tannin

Table (3) : Effect of Diets supplemented with dried bitter apple on body weight status of Steatohepatitis rats

Parameters / Groups	IBW (g)	FBW(g)	BWG%	FI (g/d/rat)	FER
Control (-ve)	192.33±0.47^{a}	201.53±1.42^{c}	4.78±0.78^{c}	16.0	0.013±0.02^{c}
Control (+ve)	192.00±1.22^{a}	170.46±1.11^{d}	-11.21±0.08^{d}	13.0	-0.037±0.004^{d}
Dried bitter apple 5%	190.00±1.08^{a}	197.66±1.31^{c}	4.03±0.10^{c}	14.0	0.012±0.004^{c}
Dried bitter apple 7%	190.83±0.77^{a}	212.30±1.43^{b}	11.26±1.16^{b}	14.8	0.032±0.03^{b}
Dried bitter apple 10%	191.66±0.84^{a}	221.13±2.36^{a}	15.36±0.72^{a}	15.5	0.042±0.02^{a}

Data are expressed as mean ± SE.

Means with different letters in the same columns are significantly different at (P<0.05)

Results illustrated in Table (3) showed the effect of diet supplemented with dried bitter apple on body weight status of steatohepatitis rats. There were no significant changes in the IBW among all groups of rats. The FBW, BWG% and FER were significantly (P< 0.05) lowered at the positive control group compared to the negative control one. However, diet supplemented with dried bitter apple at different levels significantly (P< 0.05) increased the FBW BWG% and FER compared to the positive control group. There was a significant difference of FBW BWG% and FER among the three tested groups. It was also observed the highest BWG% was recorded at the group that fed on diet supplemented with bitter apple at 10%. Moreover, the mean FI was increased at all different treated groups compared to the positive control group.

Table (4): Effect of diets supplemented with dried bitter apple on serum MDA and CAT of steatohepatitis rats

Parameters / Groups	MDA (nmol/ml)	CAT (μ/L)
Control (-ve)	3.13±0.10^{d}	136.36±0.60^{a}
Control (+ve)	7.16±0.16^{a}	70.50±2.65^{e}
Dried bitter apple 5%	4.90±0.18^{b}	92.76±2.04^{d}
Dried bitter apple 7%	4.38±0.20^{c}	112.46±1.66^{c}
Dried bitter apple 10%	3.49±0.14^{d}	126.03±2.20^{b}

Data are expressed as mean ± SE.

Means with different letters in the same column are significantly different at (P<0.05).

Table (4) showed the effect of diet supplemented with different levels of dried bitter apple on serum MDA and CAT in rats with induced steatohepatitis. MDA was significantly (P< 0.05) increased, while serum CAT was significantly decreased in the positive control compared to the negative control. Diets supplemented with bitter apple at different levels showed significant decrease (P< 0.05) in the mean level of MDA, while serum CAT was significantly (P< 0.05) increased as compared to the positive control. There was a significant differences at serum MDA and CAT among the three tested groups. It was noticed that the lowest value of MDA and the highest CAT levels were recorded for the group that fed on diet supplemented with 10% dried bitter apple.

Table (5): Effect of Diets supplemented with Bitter apple on liver functions of rats with induced Steatohepatitis

Parameters / Groups	AST (µ/L)	ALT (µ/L)	ALP (µ/L)
Control (-ve)	79.19±2.27^{d}	25.10±0.81^{d}	65.76±2.20^{e}
Control (+ve)	124.00±1.16^{a}	43.33±1.43^{a}	113.60±1.66^{a}
Dried bitter apple 5%	93.30±1.55^{b}	36.20±1.30^{b}	92.04±0.84^{b}
Dried bitter apple 7%	86.16±1.61^{c}	31.23±1.24^{c}	85.86±2.82^{c}
Dried bitter apple 10%	77.20±1.01^{d}	26.60±0.61^{d}	76.86±1.14^{d}

Data are expressed as mean ± SE.

Means with different letters in the same column are significantly different at (P<0.05).

Results in table (5) revealed the effect of different levels dried bitter apple on liver functions in rats with induced steatohepatitis. The positive control group had significant increase (P< 0.05) in the mean value of serum AST, ALT and ALP compared to the negative control group. On the other hand, the supplementation with different levels (5, 7 and 10%) of dried bitter apple significantly decreased (P< 0.05) the mean values of serum liver enzymes compared with the positive control group. There was a significant difference at serum ALT, AST and ALP among the three tested groups. The best values were obtained for liver functions levels at the group that fed on diet supplemented with dried bitter apple at 10%.

Table (6): Effect of Diets supplemented with Bitter apple on serum lipid profile in rats with induced Steatohepatitis

Parameters / Groups	TC	TG	VLDL-C	HDL-C	LDL-C
	(mg/dl)				
Control (-ve)	73.46±1.42^{d}	41.66±0.62^{e}	8.33±0.12^{e}	50.60±0.72^{a}	14.53±0.82^{c}
Control (+ve)	104.36±3.07^{a}	80.80±1.18^{a}	16.16±0.23^{a}	22.80±0.53^{e}	65.40±2.43^{a}
Dried bitter apple 5%	94.06±1.04^{b}	71.80±1.17^{b}	14.36±0.23^{b}	33.26±0.96^{d}	41.50±0.84^{b}
Dried bitter apple 7%	84.43±0.57^{c}	67.53±1.29^{c}	13.50±0.25^{c}	38.20±1.10^{c}	37.66±1.35^{b}
Dried bitter apple 10%	71.90±1.45^{d}	53.83±0.93^{d}	10.76±0.18^{d}	46.00±1.63^{b}	15.13±2.88^{c}

Data are expressed as mean ± SE.
Means with different letters in the same column are significantly different at (P<0.05).

Table (6) show the effect of diet supplemented with dried bitter apple at different levels lipid profile in rats with induced steatohepatitis. The positive control group had significant increase (P< 0.05) in the mean levels of TC, TG, VLDL-C and LDL-C , while serum HDL-C was significantly decreased when compared to the negative control group. Diets supplemented with dried bitter apple at different levels showed significant decrease (P< 0.05) in the mean levels of TC, TG, VLDL and LDL, while serum HDL-C was significantly increased (P< 0.05) as compared to the positive control group. There was a significant difference at serum TC, TG, VLDL-c and HDL-c among the three tested groups. It was observed that the more improvement of lipid profile was recorded at the group that fed on diet supplemented with bitter apple at 10%.

Discussion

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc **(Gordon and David ,2001).** Medicinal plants have been used in healthcare for a long time, to prevent and treat illness **(Dhama et al., 2018; Bilal et al., 2021; Reda et al., 2021 and Saeed et al., 2021)** as well as antidiabetic, rheumatism, snakebite, anti-tumor and insecticide **(Newman et al., 2000),** due to the presence of various complex chemical substances **(Karthikeyan et al., 2009), such** as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils **(Sharafzadeh and Alizadeh, 2012)**.

Bitter apple (Citrullus colocynthis) contains active substances such as saponins, alkaloids and glycosides **(Abdel-Hassan et al., 2000),** it is a desert plant that contains essential oils, glycosides, flavonoids, alkaloids, and fatty acids. The fruit has been studied extensively for its antimicrobial, antioxidant, and anti-inflammatory activities **(Al-Sanafi et al., 2006; Hussain et al., 2014 and Kamran et al., 2018).** C colocynthis acts as hepatoprotective Activity **(Hyderi et al., 2015).**

Bitter apple supplementation significantly improved the liver function of steatohepatitis rats, these results are in agreement with the finding **(Dar et al., 2012)** who mentioned that ethanolic extract of C. colocynthis leaves (200 mg/kg BW) showed hepatoprotective effects in vivo that could be attributed to cell membrane stabilization and hepatocyte regeneration**. Ebrahimi et al., (2016)** also found that the hydro-alcoholic extract of C. colocynthis leaves (75 mg/kg BW orally for 3 weeks) had good anti-hyperglycemic, anti-hyperlipidemic and hepatoprotective effects. The same results are also obtained by **(Tabani et al., 2018).**

The present study reported a significant reduction in lipid profile in adult male rats that fed different levels of bitter apple, this result agrees with many studies such as **Daradka et al., (2007)** who showed hypolipidemic effects of C. colocynthis L. in rabbits with a decrease in cholesterol, phospholipids, and triglyceride levels. The presence of high amounts of saponins in C. colocynthis might contribute to the reduction of cholesterol levels by reducing the absorption of cholesterol, increasing diarrhea due to increase peristalsis **(Milgate and Roberts, 1995).**

On the other hand, oxidative stress can lead to a variety of metabolic problems including formation of ROS and a weakening of antioxidants' protective effects **(Okafor et al., 2011).** The methanolic fruit extract of C. colocynthis was found to be a good antioxidant. It exhibited good free

radical scavenging activity due to the presence of gallic acid, a phenolic compound **(Kumar et al., 2008).** Phytochemical screening of C. colocynthis extracts revealed that the natural compounds in it act as an excellent antioxidant **(Benariba et al., 2013).** C. colocynthis oil can boost the function of antioxidant enzymes and protect the liver from injury **(Amamou et al., 2015).** An in-vitro study states that C. colocynthis can prevent the damage caused by free radicals to the body **(Rizvi et al., 2018).**

Conclusions

Dried bitter apple reduced the steatohepatitis status induced in adult male albino rats due to its antioxidant and anti- inflammatory effects. The best improvement of liver enzymes was observed at the group that fed on diet supplemented with dried bitter apple at 10%. So, Dried bitter apple might be benefits to patients who suffer from liver diseases.

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