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HYPOLIPIDAEMIC EFFECT OF COLD AQUEOUS EXTRACT MIXTURES OF GRAPE (*VITIS VINIVERA*) SEED, BASIL *(OCIMUM BASILICUM)* LEAVES AND MORINGA (*MORINGA OLEIFERA)* LEAVES IN RATS

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ABSTRACT: ***Different mixtures of (Moringa olifera leaves. Ocimum basilcum leaves and Grape seeds) as Mix A (Moringa leaves. basil leaves and Grape seeds), Mix. B (Moringa leaves and basil leaves), Mix. C Moringa olifera leaves and Grape seeds) and Mix D (basil leaves and Grape seeds) were analyzed for its total phenolic, total flavonoids contents, phenolic and flavonoids compounds then oral administration different mixtures with (200 and 100bwmg/k) to 60 sixty albino hypercholesterolemic rats aiming to improve their lipid profile. By the end of the experiment (28days) TC, TG, LDL, HDL, atherogenic indices (AC, CRR, AI) were analyzed. Mix A contained Total phenolic and Total flavonoid 1.23 and 0.07, Mix B 1.06 and 0.07, Mix C 1.46 and 0.09, Mix D 1.16 and 0.06 respectively. The highest total phenolic content and flavonoid in Mix C (1.46mg GAE g−1 DW) and flavonoid content (0.0068 mg QE). Narinigin the major compound flavonoid of Mix A. Mix B and Mix C as 2165.36 ppm, 9201.27ppm and2978.04 ppm. While Hesperidin 910.17ppm major compound flavonoid of Mix D. The total cholesterol, triglyceride and LDL ratios were improved by 14.98%, 36.93% and 25.09% in groups were treated with Mix A (200mg/kbw) compared with the positive control group. Significant high density lipoprotein (HDL) cholesterol and atherogenic indices (AC, CRR, AI), implying that hypochol-esterolemic effects of Mix. A 200mg/bw of rats were partly attributed to the reduced absorption of lipid and cholesterol.***

Key words: ***Moringa – Basil leaves – Grape seeds- Hypercholesterolemia - Total Phenolics – Total Flavonoids – Phenolics and Flavonoids Compounds***

INTRODUCTION

Hypercholesterolemia is a common clinical metabolic and/or genetic disorder that promotes functional and structural vascular wall injury (Napoli and Lerman, 2001). The world health report estimates that worldwide about 8% of all disease burdens in developed countries is caused by raised cholesterol levels in excess of the theoretical minimum, 3.8mmol/L (WHO, 2002). The underlying mechanisms for these deleterious effects involve a local inflammatory. Response and release of cytokines and growth factors (Napoli and Lerman, 2001). Total cholesterol levels above 200 mg/dl have repeatedly been correlated as an independent risk factor for development of peripheral vascular (PVD) and coronary artery disease (CAD)(Stapleton *et al*., 2010), thus, caused about 46% hypercholesterolemia treatment options that have become widely used, including pharmaceutical therapies which can decrease circulating cholesterol by preventing either its formation in the liver or its absorption in the intestine, also have pleiotropic effects witch can directly improve peripheral vascular outcomes (Stapleton *et al*., 2010). Moreover, drugs are not available over the counter and cannot be used for general health maintenance. Thus, increasing interests have drawn towards safe alternative

products derived from natural bioresources (Thilakarathna and Rupasinghe, 2012). A lot work has been carried out by researches on various plants to evident their effectiveness in hypercholesterolemia (Harikumar *et al*., 2013). The aim of this study was to assess the effect Different mixtures of (grape seed, *Moringa Oleifera* Lam and *Ocimum basilicum* leaves) cold extract on Hypolipidaemic .

MATERIALS AND METHODS

Materials

1. Plant samples:

*G*rape (*Vitis vinivera*) seeds By-product were obtained from jankliz Apo Elmtamer Elbhera Governorate; Basil *(Ocimum basilicum)* leaves were obtained from Faculty of Agriculture Tanta University in 2016. Moringa *(Moringa oleifera)* leaves obtained From EL-maghara north sine in July 2016.

2. Reagents:

Cholesterol powder cellulose, vitamins mixture and minerals mixture were obtained from El Gomhorya Company, Cairo, Egypt. All analysis kits were purchased from Bio diagnostic Co., Giza, Egypt.

3. Experimental Animals

Sixty six adult male albino rats of Sprague Dawley strain weighing (150±5 g) were obtained from Research Institute of Ophthalmology, Animal House Department, Giza, Egypt.

Methods

1. Preparation of Moringa leaves, basil leaves and grape seeds powders

The leaves fresh of (Moringa, basil) and grape seeds were cleaned under running tap water to remove dirt and soil residues. Then it was dried at 40˚C in an air draught oven and grinded to a fine powder and kept in dark glass bottle in deep freezer (-16 ºC) for further analysis.

2. Extraction of Moringa leaves, basil and grape seeds powders

Powder (20g) was extracted with 400 ml (hot and cold) water solvent the flask in shaking incubator at 45º C for 10 min 2h then left at room temperature 25º C ± 2 as for 24 hrs the filtrate was separated using Whitman quantitative filter paper No.42, the residual of each tested plant material, another 400 ml of the same solvent was added and left to another 24 hrs, and filtered. The extract was concentrated to dryness under reduced pressure and controlled temperature (45 - 50ºC). The yield (w⁄ w) of the extract from fresh leaves was about 8–9%. from each plant the extract was prepared in duplicate, and all analysis was carried out in triplicates.

3. Chemical analysis

3.1. Chemical composition:

The samples were analyzed for the extraction procedure used for the determination of total phenolic and total flavonoids was adapted from Chun *et al*. (2003) and Franke *et al*. (2004).

3.2. Antioxidant activity:

The DPPH assay described by Mandic *et al*. (2009) was utilized with some modifications. The stock reagent solution (1×10^{-3} mol L^{-1}) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at −20 ◦C until use. The working solution (6×10^{-5} mol L^{-1}) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8±0.02 at 515 nm, as measured using a spectrophotometer. Solutions of different concentrations (0.1 mL of each) were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min, after

which the absorbance at 515 nm was recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follows:

Radical scavenging activity% =

$$[(A^{control} - A^{sample}) / A^{control}] \times 100$$

Where: *A* is the absorbance at 515 nm.

3.3. Determination of Total Phenolic:

Total phenolic content was determined by Kaur and Kapoor (2002) A 20 µL aliquot of extract solution or processed jam (1 g 10ml methanol) solution was mixed with 1.16 mL of distilled water and 100 µL of Folin–Ciocalteu's reagent followed by 300 µL of 200 g L−1 Na2CO3 solution. The mixture was incubated in a shaking incubator at 40 ◦C for 30min and its absorbance at 760 nm was measured. Gallic acid was used as standard for the calibration curve. Total phenolic content expressed as Gallic acid equivalent (GAE) was calculated using the following linear equation based on the calibration curve: $A = 0.98C + 9.925 \times 10{-}3$ ($R2 = 0.9996$). where *A* is the absorbance and *C* is the concentration (mg GAEg−1 DW).

3.4. Determination of total flavonoids

Total flavonoid content was determined by the method of Chang *et al*. (2002) 0.5mL aliquot of 20 g L−1 AlCl3 ethanolic solution was added to 0.5 mL of extract solution. After 1 h at room temperature the absorbance at 420 nm was measured. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg mL−1. Total flavonoid content expressed as quercetin equivalent (QE) was calculated using the following equation based on the calibration curve: $y = 0.0255x$ ($R2 = 0.9812$) where *x* is the absorbance and *y* is the concentration (mg QEg−1 DW).

4. Biological investigations

4.1. Experimental animals

The work was carried out at Research Institute of Ophthalmology, Animal House Department, Giza, Egypt. Sixty-six male albino rats (150g ±5) were fed a standard diet for 7 days as an adaptation period. The standard diet was formulated according to AIN-93 guidelines Reeves *et al*., (1993) as shown in Table, (1). Salt mixture and vitamins mixture were prepared according to Hegested (1941) and Campbell (1963) as shown in Table (II) and (III). The rats were housed individually in wire cages under the normal laboratory conditions. Every day the rats were observed for the external appearance, shape, color and distribution of hair and physical activity. The diets were introduced to rats in special food cups to avoid loss of food and contamination. Also water was provided to rats by glass tube projecting through wire cages from inverted bottles supported to one side of the cage. Food and water provided were checked daily.

4.2. Experimental groups:

Male albino rats were divided randomly into two main groups, the first, negative control group (normal) (n= 6), fed on basal diet and the second group (hypercholesterolemic) (n=60), was fed on diet containing cholesterol (1.5%) and bile salts (0.25%) for 21 consecutive days to achieve hypercholesterolemia. Then, the hypercholesterolemic group (n=60) was divided into ten groups, 6 rats each. First group (positive control group) was fed on basal diet, the second was treted with drug simvaststin 3mg/kg. third and fourth groups were oral administration Mix A with (100 and 200 mg/k.bw) respectively, the fifth, sixth were oral administration Mix S with (100 and 200 mg/k.bw) respectively, seventh, eighth were oral administration Mix C with (100 and 200 mg/k.bw) respectively. And the

ninth, tenth were oral administration Mix C with (100 and 200 mg/k.bw) respectively.

4.3. Blood collection:

At the end of experimental period (30 days), rats were anesthetized with diethyl ether after fasting for 12h and blood samples were collected by using the retro-orbital method by means of a micro capillary glass tubes. Blood was collected into a dry clean centrifugal tube and left to clot in a water bath (~25°C) at room temperature for 0.5h. The blood was centrifuged for 10 min at 3000 rpm to separate the serum. Serum was kept in clear quit fit plastic tubes and stored at -20°C until analysis.

4.4. Biochemical assays

The serum triglycerides (TG), high density lipoprotein (HDL) and total cholesterol (TC) were determined according to the methods described by Fossati and Prencipe, (1982); Demacker *et al*., (1980) and Covaci *et al*., (2006). The determination of low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were carried out according to the method of Lee Castellii, (1977) as follows: VLDL= TG/5. LDL = Total cholesterol - (HDL+VLDL).

Atherogenic indices [(cardiac risk ratio (CRR), atherogenic coefficient (AC) and atherogenic index (AI)] were determined according to (Kikuchi-Hayakawa *et al.,* 1998; Dobia´sˇova´ and Frohlich, 2001).

2.5. Statistical analysis:

All experiments were performed in five replicates. The data were recorded as means ± standard deviations and analyzed with SPSS (version 12.0 for Windows, SPSS Inc., 223 South Wacker Drive, Chicago, USA). Differences were considered significant at *P* <0.05 (Keppel, 1991).

RESULTS AND DISCUSSION

1. Total phenolic and total flavonoids of cold water extract of different mixtures of moringa leaves, basil leaves and grape seeds

Table 1 showed The Effect of cold water extract on total phenolic and flavonoid of different mixture of Moringa leaves, basil leaves and Grape seeds. Total phenolic and flavonoid in Mixture of Moringa leaves, basil leaves and Grape seeds (Mix A), mixture of Moringa leaves and basil leaves (Mix B), mixture of Moringa leaves and Grape (Mix C) and mixture of basil leaves and Grape seeds showed are no different significantly at (P≤0.05). While the lowest Total phenolic and flavonoid in Mix D mixture of basil leaves and Grape seeds.

Table 2 showed the effect of cold water extract on antioxidant of different mixture of Moringa leaves, basil leaves and Grape seeds. The highest Antioxidant were (Mix A) mixture of Moringa leaves, basil leaves and grape seeds followed by (Mix C) mixture of Moringa leaves and Grape seeds and (Mix B) mixture of Moringa leaves and basil leaves. While the lowest antioxidant Mix D mixture of basil leaves and Grape seeds.

These values in the same range as the values obtained by castillo-lópez *et al*. (2017) who stated that Moringa leaves contained DPPH was 86.82 and 87.92%, respectively. Meanwhile, basil leaves. Katsube *et al.* (2004) who reported that basil leaves ranged from 58.43% - 92.37% DPPH, respectively. On the other hand Grape seeds reported by *Sonja et al*. (2009) who reported that grape seeds *Italian and Župljanka* 79 % and 95 % mg dry seeds sample/mg DPPH radical. These results agree with our results.

Table (1): Effect of aqueous extract on total phenolic, flavonoid on mixes of moringa leaves, basil leaves and grape seeds.

Prompter / Groups	Mix A Mean ±SD	Mix B Mean ± SD	Mix C Mean ± SD	Mix D Mean ± SD
Total phenolic	0.5674±0.12	0.6728±0.2	0.5128±0.16	0.2044±0.008
Total flavonoid	0.0492±0.008	0.0603±0.018	0.0492±0.008	0.0137±0.003

Mix A: (Moringa, basil and grape seeds) extracts (1 :1: 1), Mix B (Moringa and basil) extracts (1: 1), Mix C (Moringa and grape seeds) extracts (1:1), Mix D (basil and grape seeds)extracts (1:1).

Table (2): Antioxidant activity of aqueous (cold) extracts of moringa leaves, basil leaves and grape seeds.

Plants	DPPH (PPM)
Mix A	80.00± 5.4
Mix B	78.00 ± 7.07
Mix C	79.17 ±5.43
Mix D	77.17 ± 1.64

The with different letters are significantly different ($p \leq 0.05$).
Mix A: (Moringa, basil and grape seeds) extracts (1 :1: 1), Mix B (Moringa and basil) extracts(1: 1), Mix C (Moringa and grape seeds) extracts (1:1), Mix D (basil and grape seeds)extracts (1:1).

2. Identification of phenolic compound of mixtures moringa leaves basil and grape seeds (Mix A, Mix B, Mix C and Mix D) cold extract by HPLC

Total Phenolic compounds Mixture of Moringa leaves, basil leaves and grape seeds (Mix A) cold water extract. (Mix A) contained Phenolic compounds, a higher amount of catechein was 206.44 ppm, Moringa leaves contain amount of catechein was 571.15 ppm, Grape seeds contain amount of catechein was 19.84ppm and basil leaves contain amount of catechein was 97.52 ppm. catechein is phenolic acid identified in water extracts of Moringa leaves, basil leaves and Grape seeds it was found to be contributed hyperlipidemia activity of plants. Our result agree with reported by Ngamukote *et al.* (2011) who showed that gallic acid and catechin major polyphenolic compounds have cholesterol-lowering activity by inhibiting pancreatic cholesterol esterase in Grape seeds extract. The concentration of phenolic compounds in cold water extract of Moringa leaves and basil leaves (Mix B) were determined (Table 3). There is a great variation in concentration of components which are identified cold water extract of (Mix B) contained 16 fractions of phenolic compounds. The highest amount of Phenolic compounds as catechein, pyrogallol and iso- ferullic as value 420.09 ppm, 223.96 ppm, and 182.52 ppm. Identified in water extracts of Moringa leaves, basil leaves it was found to be contributed hyperlipidemia activity of plants. Our result agree with reported by Gajera *et al*. (2017) and (2018) who reported that's phenolic - gallic, catechin, and ferulic acids were a highly positively

correlated with hyperlipidemia and free radical scavenging activity. In the same table (Mix C) contained the major phenolic compounds as catechein and Salicylic value as 158.79ppm and 168.84 ppm. while the lowest amounts as caffic 2.69 ppm and comarin 4.43 ppm. (Mix D) Cinnamic 186.54ppm and catechein 57.97 a major Phenolic compound of (Mix D) ppm. Our result agree with that reported by Gengaihi *et al.* (2014) who reported that catechin 20.4 ppm was the major phenolic compounds have chloesrol lowering activity. While the lowest amounts of Phenolic compounds 0.83ppm Alpha coumaric and Amino value 2.61 ppm.

Table (3): Phenolic compound of mixture of Moring*a* leaves, basil leaves and grape seeds cold water extracts on HPLC.

Phenolic compounds	Phenolic compounds (ppm)			
	Mix A	Mix B	Mix C	Mix D
Gallic	14.60	28.95	18.25	8.12
Pyrogallol	81.92	223.96	50.83	31.46
4-Aminobenzoic	10.18	27.38	8.94	2.61
Protocatchuic	29.74	76.28	27.53	17.58
Catechein	206.44	420.09	158.79	57.97
Chlorogenic	-	54.74	39.35	11.68
Catechol	25.48	62.92	18.07	19.05
Caffeine	53.45	80.58	31.62	36.50
P-OH-benzoic	48.45	102.63	34.87	19.58
Caffeic	18.27	45.39	2.69	3.03
Vanillin	7.43	38.42	5.79	8.98
P-coumaric	7.95	21.02	4.56	7.01
Ferulic	7.33	44.08	12.25	5.26
Iso –Ferulic	66.86	182.52	66.01	1.59
Alpha- coumaric	6.42	24.04	6.86	0.85
Benzoic	65.57	167.75	37.78	36.11
Salycillic	196.64	146.71	168.84	20.71
3,4,5 –methoxy-cinnamic	12.01	19.89	5.49	5.11
Coumarin	14.40	19.38	4.43	13.76
Cinnamic	0.58	147.53	44.64	186.54

3. Identification of flavonoid compounds in different mixtures (Mix A, Mix B, Mix C and Mix D) cold extract by HPLC.

Identification of Mix A flavonoid compounds by HPLC. Table (4) shows that (Mix A) contained a higher amount of Narinigin, Rutin and luteolin value as 2165.36ppm, 1941.08 ppm and 206.42ppm respectively. Our result agrees with reported by Ding *et al.* (2012) who reported that Hesperidin reversed the hyperglycemia and hyperlipidemia by down regulating free radical generation. Priscilla*et al.* (2015) who showed that Naringenin inhibited the intestinal α-glycosidase activity thereby decreasing lipid profile changes, improved antioxidant status and hepatic function markers. Hao *et al*. (2012) reported that Rutin was demonstrated to have protective effect against dyslipidemia induced nephropathy, neuropathy, liver damage. Whilemean the Mix B contained a higher amount Narinigenin, Rutin and quercetrin 9201.27ppm, 5470.07ppm and 466.62 ppm respectively of flavonoid compounds. In concert the lowest amounts of flavonoids value as Hesprtin and Apegnin 16.60 and 23.33 ppm. Our result agree with that reported by Panda and Kar (2007) who reported that Apigenin was efficient in overcoming hyperlipidemia and reduced antioxidants like SOD, CAT, GSH in cholesterols induced hyperlipidemia rats. It also efficacy in glucose lowering. Hesperidin reversed the hyperglycemia and hyperlipidemia by down regulating free radical generation Ding *et al*. (2012). Whilmeam (Mix C) contained the highest amounts of flavonoid compounds as Narinigenin 2978.04 ppm and Hesbirdin 1959.84 ppm. Ding *et al*. (2012) who showed that Hesperidin reversed the hyperglycemia and hyperlipidemia by down regulating free radical generation. Priscilla *et al.* (2015) Reported that Naringenin inhibited the intestinal α-glycosidase activity thereby decreasing the levels of lipid profile changes, improved antioxidant status and hepatic function markers.

Mix D contained a higher amount of as Hesbirdin 910.17 ppm and luteolin 29.42ppm, Our results agree with reported by Grassi *et al.* (2008) who examined that hyperlipidemia herbs and their compounds were proven to significantly hyperlipidemia. Flavonoids form the biggest family of polyphenolic herbal compounds which are being demonstrated to possess anti-hyperglycaemic and anti-hyperlipidemic.

4. Effect of feeding on different mixtures of Moringa leaves, basil leaves and grape seeds cold water extract (100 and 200mg/kg) on triglycerides and total cholesterol in hyperlipidemic rats

The results in Table (5) showed the level of triglycerides and cholesterol in control and experimental rats as well as the reduction rate occur via treating with mixture of moringa leaves, basil leaves and grape seeds cold water extracts .The results showed significant ($p \leq 0.05$) increase of lipids profile (TC and TG) level in all groups of chemically induced hypercholesterolemia compared with normal control. All treated showed groups an improve ($P \leq 0.05$) of lipids profile level (TC and TG) in rats by oral administration of Mix A, MixB, Mix C and Mix D by 1oo and 200 mg/kg when compared with the control positive group. The groups treated with drug (simvastatin) showed the highest improve ($P \leq 0.05$) in level of triglycerides and cholesterol compared with all treated groups. A higher improving ($P \leq 0.05$) level of triglycerides group was treated Mix A with 200mg /kg. (63.7). While cholesterol

level showed improving in all groups compared the control positive. In contrast, the groups treated with drug (simvastatin) showed a higher improve (P≤0.05) compared with all treated groups. As well as group was treated Mix A with 200mg /kg (106.7) had no a significant (p ≤ 0.05) change on cholesterol level compared with the group treated with drug (105.6) followed by group treated with mixture of basil leaves and grape seeds by 200mg/kg (109.67). The result are agreement with that reported by Ganjali *et al* (2012) who reported that the methanolic extract of grape seeds effective in the treatment cholesterol, triglyceride, This effect may be due to the presence of flavonoids and antioxidant properties of grape seeds. Reddy *et al*. (2017) who reported that the polyphenol extract of Moringa leaves has a significant cholesterol lowering effective. Ding *et al*. (2012) showed that Hesperidin reversed the hyperglycemia and hyperlipidemia by down regulating free radical generation.

Table (4): Flavonoid compound of mixtures of Moringa leaves, basil leaves and grape seeds cold water extracts on HPLC.

Flavonoid	Flavonoid (ppm)			
	Mix A	Mix B	Mix C	MixD
Apig-6-arbinose8-glactose	25.95	73.23	3.84	10.95
Aig-6-rhanmnose 8-glucose	39.35	52.19	35.57	20.50
Luteolin	206.42	711.44	206.95	29.42
Narinigin	2165.36	9201.27	2978.04	49.99
Rutin	1941.08	5470.07	495.76	42.91
Hesbirdin	1138.90	3212.63	1959.84	910.17
Apigenin7-O-neohespiroside	11.19	27.91	7.19	3.66
Quercetrin	133.74	466.62	162.56	13.74
Quercetin	10.64	35.39	8.62	6.96
Kamp.3.(2-p-comaroyl)glucose	91.69	163.19	74.28	33.59
Acacetin neo-rutiiinoside	17.65	117.94	14.98	17.45
Naringenin	2.65	1.15	0.71	0.57
Hespirtin	10.69	16.60	4.54	2.79
Kampferol	14.79	--	17.24	15.19
Apegnin	12.33	23.23	7.18	11.57

Table (5): Effect of cold water extracts different mixtures of Moringa leaves, basil leaves and grape seeds cold water extract (100 and 200mg/kg) on triglycerides and total cholesterol of hyperlipidemic rats

Parameters / Groups	Triglycerides	Cholesterol
	Mean ± SD	Mean ± SD
Control Negative	43.7±2.08^{e}	102.3±2.52^{c}
Control Positive	101±2.65^{a}	125.50±3.28^{a}
G3	44.9±2.85^{e}	105.6±3.27^{c}
Mix A 100mg/bw	69.3±3.21^{c,d}	110.3±.2.52^{b,c}
Mix A 200mg/bw	63.7±1.53^{d}	106.7±3.50^{c}
Mix B 200mg/bw	84.2±1.04^{b}	117.3±2.05^{b}
Mix B 100mg/bw	81.3±2.52^{b}	115.3±2.08^{b,c}
Mix C 200mg/bw	76.3±1.52^{b,c}	110.7±2.52^{b}
Mix C 200mg/bw	73.7±2.08^{c}	113.3±3.6^{b,c}
Mix D 100mg/bw	73.7. ±2.08^{c}	109.9±2.52^{b,c}
Mix D 200mg/bw	71.0 ± 2^{c}	109.67±3.06^{b,c}

Data are presented as (Mean ± SEM), SEM = Standard error of mean.
Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

5. Effect of feeding on different mixtures of Moringa leaves, basil leaves and grape seeds cold water extract (100 and 200mg/kg) on lipoproteins of hyperlipidemic rats.

The results in Table (6) showed the activity of serum lipoprotein level of HDL and LDL activities in control and experimental rats as well as the reduction rate occur via treating with mix of moringa leaves, basil and grape seeds cold water extract on lipoproteins of rats. The results showed significant ($p \leq 0.05$) decrease of the level of lipoproteins HDL and increase of LDL in all groups of chemicallyinduced hypercholesterolemia compared with normal control. The result showed an improve (P≤0.05) in serum lipoprotein level of HDL and LDL by oral administration of Mix A, Mix B , Mix C and Mx D) by 1oo and 200 mg/kg when compared with the control positive group. In contrast, the groups treated with drug (simvastatin) showed a higher improve (P≤0.05) in HDL and LDL level compared with all treated group. The highest improve (P≤0.05) of HDL was detected in the group treated with Mix A extracts by 100mg and 200mg /kg 43.07

and 44.40 as well as the groups treated with drug (simvastatin) 45.67 had no a significant (P≤0.05) changes compared with all treated groups. Meanwhile, The LDL level was observed in all groups treated with Mix A, Mix B, Mix C and Mix D by 100mg and 200mg/kg had no a significant (P≤0.05) changes compared with group positive. The best result of LDL groups treated with drug (simvastatin). Our result agree with that reported by Souravh *et al*. (2014) who reported that, moringa leaves effective in the treatment HDL, LDL of hypercholesterolemia. This effective may be due to the presence of total phenolic and flavonoids of moringa leaves. Ganjali *et al.* (2012) showed that the methanolic extract of grape seeds effective in the treatment HDL, VLDL of hypercholesterolemia. This effect may be due to the presence of flavonoids and antioxidant properties of grape seeds. Hao *et al.* (2012) reported that Rutin was demonstrated to have protective effect against hyperglycemia and dyslipidemia induced nephropathy, neuropathy, liver damage. Priscilla *et al*. (2015) reported that Naringenin inhibited the intestinal α-glycosidase activity thereby decreasing the levels lipid profile, improved antioxidant status and hepatic function markers.

Table (6): Effect of cold water extracts different mixtures of moringa leaves, basil leaves and grape seeds 100 and 200 mg/kg on lipoproteins in hyperlipidemic rats.

Parameters / Groups	HDL	LDL	VLDL
	Mean ± SD	Mean ± SD	Mean ± SD
Control Negative	46.67±1.53^{a}	47.3±2.08^{d}	8.73±0.41
Control Positive	31±0.50^{d}	71.9±1.79^{a}	30.26±17.95
G3	45.33±2.08^{a}	49.9±2.80^{d}	8.98±0.73
Mix A 100mg/bw	43.07±1.26$^{a.b}$	58.3±3.01^{b}	12.73±0.30
MixA200mg/bw	44.40±1.44^{a}	53.9±2^{c}	9.86±0.64
Mix B 200mg/b	34.17±1.72^{c}	63.3±1.2^{b}	16.83±0.20
MixB 100mg/bw	36.07±2.10c	61.3±1.53^{b}	16.26±0.50
MixC 200mg/bw	37.93±1.06$^{b.c}$	60.5±2.52^{b}	45.8±0.30
MixC 200mg/bw	39.20±1.06^{b}	58.0±1.35^{b}	8.8±0.41
Mix D 100mg/bw	40.43±1.00^{,b}	59.3±2.52^{b}	14.73±.41
Mix D 200mg/bw	42.00±1.69^{a,b}	56.2±1.37^{b,e}	14.2±0.4

Data are presented as (Mean ± SEM), SEM = Standard deviation of mean.
Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

6. Effect of feeding on different mixtures of Moringa leaves, basil leaves and grape seeds cold water extract (100 and 200mg/kg) on AI. CRR. CRI. AF and AC of normal and hyperlipidemic rats.

Data in Table (7) showed the effect of mixs of moringa, basil and grape seeds on AI. CRR. CRI .AF and AC of normal control and hypercholesterolemia rats. The results in Table (7) showed the level of (AI.CRR.CRI.AF and AC) all groups were improving compared the control positive all groups reduced (AI.CRR.CRI.AF and AC) level compared positive control. In contrast, the groups treated with drug (simvastatin) showed a higher improved compared with all treated groups. The best result in group was treated with Mix A and Mix D 100mg and 200mg /kg of body weight. The level of atherogenic coefficient (IA) was improved when the hypercholesterolemic rats that administrated with Mix A and Mix D (100mg and 200mg /kg bw) and Mix C with 200mg/k bw compared to positive control group. The best hypercholesterolemic group in cardiac risk ratio (CRR and AC) was that administrated with Mix A and Mix D (100mg and 200mg /kg of bw) and Mix C with 200mg/k bw while the lowest improvement was in Mix B100mg and 200mg /kg of bw) and Mix C with 100mg. 0n 0nther hand The level of atherogenic coefficient (CRI and FA) was improved when the hypercholesterolemic rats that administrated with Mix A and Mix D (200mg /kg bw). Atherogenic indices are powerful indicators of the risk of heart disease the higher the value, the higher the risk of developing CVD and vice versa (Usoro *et al.,* 2006 and Krishna, 2013).

7. Effect of feeding on different mixtures of Moringa leaves, basil leaves and grape seeds cold water extract (100 and 200mg/kg) on antioxidant activity of normal and hyperlipidemic rats.

Table (8) revealed the effects of feeding mixture of moringa leaves, basil leaves and grape seeds extracts on (SOD - MDA) in hyperlipidemia rats. The results showed the activity of liver tissue oxidative marker (MDA, and SOD activities) in control and experimental rats as well as the reduction rate occur via treating with mix of moringa leaves, basil leaves and grape seeds extracts. The results showed significant ($p \leq 0.05$) increase of oxidative marker MDA, and decrease of SOD in all groups of chemically induced hypercholesterolemia compared with normal control. All groups rats treated showed improving ($P \leq 0.05$) of MDA, and SOD activities when compared control positive group by oral administration of Mix A, MixB, MixC and MixD with 1oo and 200 mg/kg when compared with the control positive group. In contrast, the groups treated with drug (simvastatin 3mg/kg) showed a higher improve ($P \leq 0.05$) compared with all treated groups. The groups treated with Mix A cold extracts by 200mg /kg as well as the groups treated with drug (simvastatin) had no a significant ($P \leq 0.05$) changes of level MDA. While the best result of SOD level groups treated with Mix A flowed to Mix C and Mix D by 100 and 200 mg/kg showed a higher improving ($P \leq 0.05$) when compared with the control positive group. In contrast, the groups treated with drug (simvastatin) showed a higher improve ($P \leq 0.05$) compared with all treated groups. This result agree with that reported by Reddy *et al. (*2017) who

reported that Treatment with Moringa leaves extract reduce the level of MDA and increase of SOD. Sakr and Al-Amoudi (2012) reported that basil leaves cold water extract Reduction in the level of MDA (lipid peroxidation marker) and increase in the activities of SOD. The present investigation suggested that moringa leaves, basil leaves and grape seeds cold extracts had a potential role in therapeutic action via inhibiting oxidative stress due to presence of phenolic compounds and its anti-oxidant nature that s result agree with that reported by Jaiswal *et al*. (2013) who reported that moringa leaves extracts increasing SOD, CAT, GSH and decreased level lipids in hyperlipidemia rats.

Table (7): Effect of cold water extracts different mixtures of moringa leaves, basil leaves and grape seeds extracts 200 mg/kg on lipids profile of hyperlipidemic rats

Parameters / Groups	AI	CRR	CRI	FA	AC
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control Negative	0.93±0.25^{f}	2.19±0.55^{e}	1.02±0.75^{f}	0.46±0.01^{a}	1.19±0.55^{e}
Control Positive	3.24±0.13^{a}	4.03±0.17^{a}	2.31±0.10^{a}	0.25±0.01^{e}	3.03±017^{a}
G3	0.99±0.10^{f}	2.34±0.13^{e}	1.10±0.30^{ef}	0.43±0.26^{ab}	1.34±0.17^{e}
Mix A 100mg/bw	1.62±016^{e}	2.5±0.10^{e}	1.36±0.65^{d}	0.39±0.01^{b}	1.56±0.100^{e}
MixA200mg/bw	1.43±0.75^{d}	2.40±0.05^{de}	1.22±0.72^{de}	0.42±0.00^{b}	1.40±0.005^{de}
Mix B 100mg/bw	2.46±0.75b	3.44±0.17^{b}	1.85±0.10^{b}	0.29±0.02^{e}	2.44±0.13^{b}
MixB 200mg/bw	2.26±0.20^{b}	3.12±0.17^{b}	1.70±0.07^{b}	0.32±0.15^{d}	2.12±0.17^{b}
MixC 100mg/bw	2.01±0.85^{c}	3.04±0.8^{c}	1.60±0.61^{c}	0.33±0.01^{d}	2.04±0.83^{c}
MixC 200mg/bw	1.87±0.86^{c}	2.83±0.15^{c}	1.48±0.05^{c}	0.35±0.02^{c}	1.83±0.15^{c}
Mix D 100mg/bw	1.82±0.10d	2.81±0.16^{d}	1.47±0.04^{cd}	0.35±0.02^{c}	1.81±0.16^{cd}
Mix D 200mg/bw	1.67±0.68^{c}	2.61±0.08^{cd}	1.34±0.03^{d}	3.80±0.01^{c}	1.61±0.08^{cd}

Means in the same row with different letters are significantly different (p < 0.05). G3: rats treated with simvastatin3mg/kg. Mix A (Moringa leaves , basil leaves and grape seeds) extracts (1 :1: 1), Mix B ((Moringa leaves and basil leaves) extracts(1: 1), Mix C (Moringa leaves and grape seeds) extracts (1:1),Mix D (basil leaves and grape seeds)extracts(1:1) Data presented are in means ± standard deviations for five replicates in each group. Values in the same row not sharing a common superscript letter differ significantly at P < 0.05. * CRR= Cardiac risk ratio, (AC) = Atherogenic coefficient, AF =Atherogenic Fraction, AI= Atherogenic Index, CRI = Atherogenic Indix

Table (8): Effect of cold water extracts different mixtures of moringa leaves, basil leaves and grape seeds extracts on (SOD - MDA) in hyperlipidemic **rats.**

Parameters / Groups	MDA	SOD
	Mean ± SD	Mean ± SD
Control Negative	35.67±1.53^{g}	0.80± 0.05^{a}
Control Positive	2.46^{a}±81.80	0.024± 0.02^{f}
G3	36.50±1.55^{g}	0.01^{b}±0..60
Mix of E (M+G) 100mg/bw	43.70±1.61^{f}	0.52± 0.03^{c}
Mix of E(M+G) 100mg/bw	38.63±1.72^{g}	0.50± 0.02^{c}
Mix of E (M+B) 100mg/bw	68.33±3.21^{b}	0.32± 0.02^{e}
Mix of E(M+B) 100mg/bw	64.05±1.29^{c}	0.34± 0.01^{e}
Mix of E (M+G)100mg/bw	56.03±2.16^{d}	0.48 ±0.01^{c}
Mix of E(M+G) 100mg/bw	51.47±1.50^{e}	0.51± 0.05^{c}
Mix of E (B+G) 100mg/bw	46.50±1.54^{f}	0.41± 0.02^{cd}
Mix of E (B+G) 200mg/bw	44.60±1.62^{f}	0.45± 0.03^{cd}
L SD	3.359	0.054

Data are presented as (Mean ± SEM).
SEM = Standard Divenation of mean.
Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

CONCLUSION

In conclusion, of mixtures of moringa, basil leaves and grape seeds were efficient in the protection against hypercholesterolemia by decreasing serum TC, TG, TI, and LDL-C and increasing HDL-C, and thus decreasing the atherogenic indices (AC, CRR, AI). Notably, the studied could be considered as potential sources of flavonoids, phenolic compounds and antioxidant activity which could be used in a wide variety of applications, mainly in the food and confectionary industries. The pharmaceutical, cosmetic and perfume industries are other possible outlets of these flavonoids, phenolic compounds and antioxidant activity.

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التأثير المثبط لدهون الدم بالمستخلصات المائية الباردة للخلطات المختلفة من أوراق الريحان وأوراق المور ينجا وبذور العنب على الفئران

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المخلص العربي

تمت هذه الدراسة بهدف دراسة تأثير المركبات الحيوية في الخلطات المختلفة والمكونة من مستخلصات أوراق المورينجا وأوراق الريحان وبذور العنب . وكانت الخلطة الأولى تتكون من مستخلصات أوراق المورينجا وأوراق الريحان وبذور العنب على تثبيط دهون الدم, والخلطة الثانية تتكون من مستخلصات أوراق المورينجا وأوراق الريحان، والخلطة الثالثة تتكون من مستخلصات أوراق المورينجا وبذور العنب، والخلطة الرابعة تتكون من مستخلصات أوراق الريحان وبذور العنب . وتم تقدير الفينولات والفلافونيدات الكلية للخلطات وكانت الخلطة الثالثة أغناهم في محتوى الفينولات والفلافونيدات الكلية ١,٤٦(mgGAEg−1 DW) – ٠,٠٩ (mg QE•g−1dw) على الترتيب.

وتبين كذلك أن النارجنينين المركب الرئيسي من المركبات الفلافونيديه للخلطات الأولى والثانية والثالثة، أما الخلطة الرابعة كان الهسبردين المركب الرئيسي فيها.

تم إعطاء ٦٠ فار الجرعات ١٠٠−٢٠٠ ملجم من الخلطات السابقة الذكر ودراسة تأثيرها على الكوليسترول الكلى والتراى جلسريد والليبوبروتين منخفض الكثافة والليبوبروتين عالي الكثافة، (AC, CRR, AI) atherogenic indices

ولوحظ من النتائج تحسن في كلاً من الكوليسترول الكلى والتراى جلسريد والليبوبروتين منخفض الكثافة والليبوبروتين عالي الكثافة وذلك في المجموعات التي تم إعطاؤها الخلطة الأولى بجرعة ٢٠٠ ملجم لكل كيلو جرام من وزن الفار مقارنة بالمجموعة الضابطة الموجبة.من خلال هذه الدراسه يتين ان تاثير المستخلصات السابقة الذكر على الكوليسترول الكلى والتراى جلسريد والليبوبروتين منخفض الكثافة والليبوبروتين عالي الكثافة، (AC, CRR, AI) atherogenic indices يرجع هذا الى الفينولات والفلافونيدات ونشاط مضادات الأكسدة للخلطات .

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