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Reducing the hypercholesterolemic effect in rats by feeding crackers blended with some selected herbs as natural coloring agents

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Abstract: The objective of this study was to evaluate the cholesterol lowering effect of some selected herbs as natural coloring agents in cracker manufacturing. The herbs used including ziziphus, curry and marjoram at concentrations of 10%. Blending of such herbs to crackers leads to significant ($p \leq 0.05$) improve in their sensory characteristics color and nutritional value. For biological evaluation, 36 mature male rats distributed into 6 equal groups. Group (1) was kept as a normal control (fed on a basal diet). Thirty rats of the other groups were fed on diet with 2% cholesterol and injected intramuscularly with 0.05 ml cholic acid for 30 days to induce hypercholesterolemia and classified into five equal groups as follow: group (2) were left as a control positive and those of groups (3, 4, 5 and 6) were given cracker (100% wheat flour C_{WF}); (C_{WF}+10% ziziphus, C_{ZP}); (C_{WF} +10% curry, C_{CP}) and (C_{WF} +10% marjoram, C_{MP}), respectively. The results showed that cracker with herbs C_{ZP} , C_{CP} and C_{MP} were significantly decreased in adiposity index (AI), serum levels of TC, TG, LDL-c ,VLDL-C, atherogenic index, uric acid, creatinine, urea nitrogen, ALT, AST, ALP, Leptin and MDA compared with the positive control group . On the other hand, there was increasing in the levels of adiponetin, serum total antioxidant capacity, superoxide dismutase and acetylcholine esterase activities compared to the positive control group. In conclusion, crackers blended with ziziphus, curry and marjoram powders could be used for the treatment of hypercholesterolemic adverse effects in rats. Like of these products will be in a high degree of importance from the nutritional and therapeutic point of views.

Keywords: Ziziphus, curry, marjoram**,** antioxidant activity, rheological properties, sensory characteristics.

Introduction

Crackers are baked foods typically made from flour. Flavorings or seasonings, such as salt, herbs, seeds, and/or cheese, may be added to the dough or sprinkled on top before baking. Crackers are a nutritious and easy way to consume a staple food or cereal grain (**Wangcharoen *et al.*, 2005; Sompong *et al.*, 2011 and Sutharut and Sudarat 2012).** However, several of them are harmful to health, as they have high fat, salt or sugar content which can cause dental caries, and increased the total cholesterol, diabetes, obesity and coronary heart disease (CHD) **(Tananuwong and Tewaruth, 2010).**

Natural coloring agents are natural pigments derived from vegetable, fruits and herbs. They are a mixture of carotenoids, anthocyanins, flavonoids, chlorophyll and other natural components of plants such vitamins, organic acids, glycosides, and aromatic substances (**Tomaska and Brooke-Taylor 2013).** They contain flavoring ingredients besides the color pigments. Coloring agents have been used for food coloring for a long time (**Newsome *et al.*, 2014)**. The Natural pigments as coloring agents are a better alternative as they are wholly derived from plants. Natural coloring usually appears less vibrant when compared to artificial coloring **(Delia and Rodriguez, 2016 and Newsome *et al.*, 2014**).

Ziziphus (*Ziziphus Jujube Mill*) belongs to the family *Rhamnaceae. Ziziphus* species are used commonly in traditional medicine to treat various diseases such as gastrointestinal disorders, fatigue, obesity, liver pain, urinary tract problem, insomnia, diabetes, loss of appetite, fever, bronchitis, diarrhea, pharyngitis and anemia (**Panahi *et al.*, 2011 and Kim *et al.*, 2013**). Ziziphus is rich in sugar, vitamin C, bioflavonoids, fibers, and minerals (**Pawlowska *et al.*, 2009**). Pharmaceutical ingredients in ziziphus are sterols, alkaloids, serotonin, saponins, flavonoids, polyphenols, and terry terpenes (**Hemati *et al.*, 2010)**. These materials have a wide range of biological activities likely antibacterial, anti-inflammatory, antiulcer, antitumor and antithrombotic (**San *et al.*, 2009**).

Curry *(Murraya koenigii*)is meaning of Thai words means sour and yellow color as pigment derived used to enhance several spicy Thai dishes (**Juntachote *et al.*, 2006)**. It has been found that flavonoids reduce

the lipid and glucose levels in blood and supports the immune system as a source of antimicrobial and antioxidant compounds (**Ahmed *et al.*, 2002).** Curry powder is a popular spice mix that has a number of worthy health benefits, such as prevention of cancer, protection against heart disease, reduce Alzheimer's symptoms, reduction inflammation, enhance bone health, protect the immune system, and increase the liver's ability to drive out toxins (**Choi, *et al.*, 2006)** .

Marjoram (*Origanum Majoranum L.*) is a member of the mint family *Lamiaceae*. Typically, products identified marjoram as the dried leaves and flowering tops of marjoram, which is found throughout the world (**Roth 2001 and Al-Howiriny *et al.*, 2009).** It contains phenolics terpenoids (thymol, carvacrol), flavonoids (diosmetin, luteolin, apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin, thymonin), triacontan, sitosterol, acids (oleanolic acid) and *cis*-sabinene hydrate **(El-Ashmawy *et al.*, 2007 and Naif 2011).**

The present study was carried out to develop high nutritious and delicious crackers through their blending with some herbs as natural coloring agents. Also, the effect of feeding such crackers on hypercholesterolemic adverse effects of rats will be in the scope of this investigation.

Materials and Methods

Materials

Herbs: Ziziphus *(Ziziphus Jujube Mill)*, Curry (*Murraya koenigii*) and Marjoram (*Origanum majoranum L.*) were purchased from Haraz Company for Herbs and Medicinal Plants, Cairo, Egypt.

Wheat and chemicals: Soft wheat flour (72% extraction) was obtained from local market in Cairo, soy flour and soy milk were obtained from Soy Factory, FTRI., ARC. Cholic acid was purchased from Sigma Chem. Co., St. Louis, Mo. Cholesterol was purchased from El-Nasser Pharm Co. Cairo, Egypt.

Animals: A total of 36 male Sprague-Dawley rats weight, 100 ±10 g were provided from of National Research Center, Cairo, Egypt. Rats were housed as groups in wire cages under the normal laboratory conditions. The basal diet prepared according to (**NRC 1995).**

Methods

Chemical analysis

Antioxidant activity: Antioxidant activity (%) was determined according to (**AOAC 2005)**.

Phenolic compounds: Phenolic compounds were determined by HPLC according to the method of **Goupy *et al.*, (1999**).

Chemical composition: Moisture content, total solids, crude fiber, ash, protein and crude ether extract were determined according to (**AACC 2000).** Total carbohydrates were calculated by the difference.

Crackers preparation

The crackers were formulated using some selected herbs powder as coloring agents ziziphus jujube, curry and marjoram by 10% to wheat flour as follow: Wheat flour by 100% C_{WF} (a) as control, C_{WF}+ 10% ziziphus (C_{ZP}) to produce red dough (b), C_{WF} +10% curry (C_{CP}) to produce yellow dough (c) and C_{WF} +10% marjoram (C_{MP}) to produce green dough (d). Other ingredients used in this additives cracker formulation were: 25 ml soy milk, 20g butter, 25g sugar, 2g salt, 3 g baking powder, and 3 ml vanilla flavor, according to the method of **Abd El Rahim *et al.*, (2003)**.

Rheological properties

The effect of blended powder of ziziphus, curry and marjoram on dough characteristics was determined by using farinograph (modle No:81010, Dusiburg, Germany) according to the methods described by **AACC (2005**). Elastic properties of dough samples different levels of flours were measured using extensograph according to the **AACC (2005)** standard methods.

Induction of hypercholesterolemic in rats

Rats were provided 2% cholesterol orally and of injected intramuscularly with cholic acid (0.05 ml /100 BW) single dose weekly for 6 weeks in an attempt to induce a case of hypercholesterolaemia as described by **Beynen *et al.*, (1986).**

Biological experiment:

Experimental design and feeding

Thirty six adult male albino rats Sprague Dawley strain with an average weight of (100 ±10 g). The rats were housed in stainless steel cages with wire mesh bottoms and maintained in temperature and humidity control with 12 hrs light / dark cycle. All rats were allowed to free access drinking of water and basal diet for seven days adjustment to the laboratory environment. Then, rats were randomly divided into 6 groups (6 rats preach) as follow:

Group (1): Negative control (ve-); fed on basal diet.

Group (2): Positive control (ve+); fed on hypercholesterolemic diet.

Group (3): Fed on hypercholesterolemic diet with control crackers 100% wheat flour (C_{WF}) diet.

Group (4): Fed on hypercholesterolemic diet blended with control crackers +10% ziziphus powder (C_{ZP})

Group (5): Fed on hypercholesterolemic diet blended with control crackers + 10% curry powder (C_{CP})

Group (6): Fed on hypercholesterolemic diet blended with control crackers + 10% marjoram powder (C_{MP}).

At the end of the experiment, body weight of rats was recorded, and euthanized by prolonged exposure to ether anesthetic. The abdomen was opened, and body fats, including mesenteric, visceral, epididymal and retroperitoneal fats were carefully dissected out and total fat mass was weighed. The adiposity index (AI) was calculated as (total body fat/final BW) × 100 as described by **Taylor and Phillips (1996).**

During the experiment period, the quantities of diet, which were consumed and/or wasted, were recorded every day. In addition, rat's weight was recorded weekly to determine feed intake (FI) and body weight gain (BWG) % according to **Chapman *et al.*, (1959).** Blood samples were collected for serum separations biochemical analyses.

Biochemical analysis

Lipids parameters were determined by enzymatic methods as follows: Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG)), according to **Richmod (1973), Lopes-Virella *et al.*, (1977), and Fossati and Prenape (1982**) respectively.

While (LDL-c and VLDL-c) were calculated according to the equation of **Friedwald *et al.*, (1972).** Atherogenic Index (Total cholesterol / HDL-cholesterol) were calculated according to the equation of **Golay *et al* ., (1990).** Uric acid was determined in the serum according to the method described by **Fossati *et al.*, (1980).** Urea nitrogen was determined according to **Patton and Crouch, (1977).** Creatinine was determined according to **Bartels *et al.*, (1972).** Serum alanine and aspartae aminotransferase (ALT, AST), alkaline phosphates (AP) enzymes, were estimated according to **Reitman and Frankel (1957), Kind and King (1954), and Weichselbaum (1946)** respectively. Leptin was measured using enzyme-linked immunosorbent assay according to **Xiong *et al.*, (2010)**. Adiponectin was chemically determined according to the methods described by **Yokota, (2000).** Superoxide dismutase (SOD) activity, total antioxidants capacity (TAC), and malondialdehyde (MDA) were determined according to **Nishikimi *et al.*, (1972), Cao *et al.*, (1993) and Ohkawa *et al.*, (1979)** respectively. Acetylcholine esterase (AChE) activity was determined colorimetrically according to **Hestrin (1949).** Isoenzymatic specturm of AChE was resolved by polyacrylamide gel electrophoresis, as develop by **Davis, (1964) and Ornstein (1964).**

Statistical analysis

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups **Snedecor and Cochran (1967).**

Results and Discussion

Antioxidant activity of the selected herbs powder

Antioxidant activity of the selected herbs powder are tabulated in Table (1). From such data it could be noticed that the all selected herbs powder of ziziphus, curry, and marjoram were exhibited high degree of antioxidant activity. With respect to potential health benefits, antioxidant activity radical in herbs have been found to make a major contribution to human health and multiple positive biological effects. Beside the health benefits, color is an important attribute which enhances the quality of orange curry. The orange color of the paste has been related to the

carotenoid pigments mainly capsanthin and capsorubin (**Surh 2002, Karakaya 2004, Willcox *et al.*, 2004 and Roy *et al.*, 2007**).

Table (1): Antioxidant activity (%) of selected herbs powder

Herbs	Ziziphus	Curry	Marjoram
Antioxidant activity radical (DPPH) (%)	92.12	92.81	85.33

Phenolic compounds of selected herbs powder

Data in table (2) indicated that the HPLC analyses indicated the presence of *P*-OH-Benzoic, epicatechen, caffeic, vanillic, ferulic, hydroxycitric acid, coumarin, ellagic, and cinnamic, as phenolic acids, the most abundant phenoilic acids in all herbs. Also, the data acids shows the presence of remark able amount of phenolic compounds in the leaves of ziziphus, curry, and marjoram as antioxidant activity and color of the herbs (**Aneta 2007 and Huang *et al.*, 2010).**

Table 2: Phenolic compounds concentration (mg/100g) in herbs powder

Phenolic compounds	**Ziziphus**	**Curry**	**Marjoram**
4-Amnobenzoic	4.77	1.14	------
Chlorogenic	---------	------	28.64
P-oH-Benzoic	59.11	4.23	27.84
Epicatechen	37.27	41.53	48.74
Caffeic	4.68	5.08	28.8
Vanillic	10.28	2.91	3.54
Ferulic	28.40	4.45	35.88
Benzoic	732.22	45.98	----
Salicylic	-----	-----	475.03
hydroxycitric acid	55.21	43.87	39.65
Coumarin	83.09	3.06	45.10
Ellagic	83.11	15.49	317.43
Cinnamic	179	4.57	49.30
Gallic acid	23	4.11	-----
Protocatechuic	24.96	3.93	-----
Catechol	------	2.08	9.6
Caffiene	14.05	----	19.78
Chlorogenic	24.56	2.38	------
Curcumin	-------	201	-----
Quercetin	49.8	----	-----
Oleanolic acid	8.36	----	----
Jujuboside A	9.501	----	1.34
Rutin	4.08	2.30	---

Chemical composition of crackers samples

The chemical composition of control and blended crackers samples are shown in Table (3). From such that it could be noticed that moisture content increased gradually by increasing level of slected herbs powder cracker which attributes their high content. Also, a high content in protein and ash blended cracker samples comparing to control one. A noticeable increase in fat and carbohydrate content as the herbs level increased in the formula. These results agree with **Zheng, and Wang (2001**) who reported that the content of the spices/herbs were characterized and their chemical composition determined were fairly high so some of them were not only used for food but also in medicine applications.

Table 3: Chemical composition of crackers samples (%)

Crackers samples	Moisture	Protein	Fat	Ash	Carbohydrate
C_{WF}	9.86	10.12	4.09	5.21	73.33
C_{ZP}	8.70	10.56	1.82	6.47	60.50
C_{CP}	8.54	11.73	2.49	5.75	66.66
C_{MP}	8.50	11.41	2.75	6.52	59.74

Values are the means of 3 independent determinations. C_{WF}: 100% wheat flour, C_{ZP}: C_{WFt} +10% ziziphus, C_{CP}: C_{WFt} +10% curry, C_{MP}: C_{WFt} +10% marjoram.

Rheological properties of dough formulae from wheat flour samples and their blended with selected herbs

Farinograph parameters data in Table (4) showed that, the wheat flour control samples absorbed 57.0% of water, when the flour of dough blended with ziziphus, curry and marjoram absorbed 68.3, 65.2 and 63.8% of water respectively. These results indicated that the increase in absorption of water as the results of herbs blending could be attributed to their high content of fiber **Peter (2004**). These results agreed with **Unver and Domolds (1976) and Ajila *et al.*, (2008)** who found that water absorption of dough increase when protein content decreased. Also, data showed that dough of wheat flour control had arrival time 0.5 and flour blended with herbs increased in arrival time which ranged from 1.0 to 1.7 min. Dough development in wheat flour control equal 1.0 min, which increased flour blends with (1.5 min). Value of dough stability for control wheat flour samples were 1.0 min which indicated to weak dough, while

value for wheat flour blends with herbs were 3.5, 2.5 and 2.5 min. for curry, ziziphus and marjoram dough respectively.

These results indicated that the flour with natural additive powders had the ability barrier to gas which effects on the freshness of the product. The degree of softening of wheat flour control were 70 B.U, which increased for flour with ziziphus and marjoram powder were 80, 90 and 110 B.U. These results indicated that the addition of powders to flour increase the degree of softening of dough. This result confirms the previous result for dough stability. From these results we can conclude that, the blended cracker with ziziphus, curry and marjoram powder will have lowest staling and lowest firmness than the control samples, because the high softening led to produce product with antistaling and low firmness. With in similar study, **Dapčević *et al.*, (2009)** reported that, degree of softening is predominantly influenced, as it was for the dough stability, by the amount and quality of gluten.

The results Table (5) showed that the extensograph parameters of dough formulae from wheat flour and samples blended with some selected herbs powder. The extensibility of dough control and dough from flour with selected herbs powder, the dough which made from flour with ziziphus, curry and marjoram powder had a lower value of extensibility than the control dough which had 195,130,160 and 140 mm, respectively. Also, the elasticity and dough resistance (R) of the control wheat flour are higher than recorded for the herbs powder blended. The present data suggested that degree of softening is predominantly influenced, as it was for the dough stability, by the amount and quality of gluten, these result agree with the result at above. These results are in parallel with those obtained by (**Ozcan 2009 and Gurpreet *et al.*, 2014).**

Table (4): Farinograph parameters of dough formulae from wheat flour and wheat flour blended with herbs powder

Samples	Water absorption (%)	Arrival time (min)	Dough development (min)	Dough stability (min)	Degree of softening (B.U)
C_{WFt}	57.0	0.5	1.0	1.0	70
C_{ZP}	68.3	1.0	1.5	2.5	80
C_{CP}	65.2	1.5	1.0	3.5	110
C_{MP}	63.8	1.7	1.0	2.5	90

C_{WF}: 100% wheat flour, C_{ZP}: C_{WFt} +10% ziziphus, C_{CP}: C_{WFt} +10% curry, C_{MP}: C_{WFt} +10% marjoram.

Table (5): Extensograph parameters of dough formulae from wheat flour and wheat flour blended with herbs powder

Samples	Extensibility (mm)	Elasticity (B.U)	proportional number (P.N)	Energy (cm^2)
C_{wf}	195	510	2.6	85
C_{ZP}	130	430	4.3	81
C_{cp}	160	460	3.8	83
C_{MP}	140	350	2.5	109

C_{WF}: 100% wheat flour, C_{ZP}: C_{WFt} +10% ziziphus, C_{CP}: C_{WFt} +10% curry, C_{MP}: C_{WFt} +10% marjoram.

Effect of prepared crackers on fats weight (FW), body weight gain % (BWG) and adiposity index (AI) of hypercholesterolemic rats

The fats weight, body weight gain ratio and adiposity index of control and blended crackers samples are shown in Table (6). From such that it could be noticed that the untreated group (+ve) feed showed significant increase ($P<0.05$) in fats weight (FW), body weight gain (BWG) and adiposity index (AI) when compared to negative control rats. The all treated groups showed significant intervening in fats weight (FW), BWG, and AI compared to positive control group. These results agree with those reported by **Yamamoto *et al.*, (2000**) who found that at obese rats fed the high-fat diet were treated with herbs induced the BW. Also, β-cryptoxanthin in curry was reported to inhibit body weight and adipocyte hypertrophy in obese rats (**Takayanagi *et al.*, 2011)**. The major groups of bioactive components in selected herbs are flavonoids, carotenoids, and phenethylamine alkaloids (**Stohs *et al.*, 2011)**

Table (6): Effect of crackers samples on fats weight (FW), body weight gain ratio (BWG), adiposity index (AI) and body mass index (BMI) of the hypercholesterolemic rats

Groups	F. wt (g/ day)	B.W.G. (%)	Ad. I (%)
Negative control	5.58±0.12^{c}	45.19 ± 4.28 ^{b}	2.79±0.10 ^{d}
Positive control	14.55±0.22 ^{a}	98.95 ± 7.89 ^{a}	4.93±0.15 ^{a}
Group 3 (C_{wf})	7.44±0.13 ^{b}	50.93 ± 4.91^{b}	4.75±0.14 ^{a}
Group 4 (C_{ZP})	7.00±0.10 ^{c}	42.65 ± 5.89 ^{c}	3.19±0.12 ^{b}
Group 5 (C_{cp})	6.71±0.10 ^{c}	43.56 ± 3.21^{c}	2.89±0.18 ^{c}
Group 6 (C_{MP})	6.70±0.11^{c}	45.01 ± 4.26 ^{c}	3.13±0.15 ^{b}

Values are expressed as means ± SD. Values with the different letters in the same column are significantly difference at $P\leq0.05$

Effect of crackers samples on lipids profile and atherogenic index of hypercholesterolemic rats

The lipids profile and atherogenic index of control and blended crackers samples are shown in Table (7). From such that it could be indicated that hypercholesterolemia have significant ($P < 0.05$) increases in serum levels of TC, TG, LDL-c and VLDL-c when compared with the normal group. However different significant decreasing on TC, TG, LDL-c , VLDL-c and atherogenic index levels were observed for the C_{WFt} C_{ZP} , C_{CP} and C_{MP} groups when compared with positive control group. The more prominent effect being observed in LDL-c which is a known triggering factor for coronary occlusion or its block. This can substantiate the cardioprotective effect of herbs. This finding is in agreement with an earlier study by **Newsome *et al.*, (2014)** who reported that several isoflavone constituents such as lowed in the selected herbs are unique phytoestrogens, which like estradiol, affect the serotonergic system, inhibiting serotonin re-uptake and thereby increasing the levels of serotonin in synaptic clefts. Also, **Zheng and Wang (2001)** reported that herbs exhibited a marked reduction in the hepatic total cholesterol and triglyceride levels both in the presence and absence of dietary cholesterol; the reduction of triglyceride levels in the absence of dietary cholesterol was in a dose-dependent manner. Finelly content of soluble fibers are non-digestible carbohydrates, which are fermented in the colon by resident anaerobic bacteria. May be to soluble fibers have effect of reduce hypolipidemic (**Atindehou *et al.*, 2004)**.

Effect of crackers samples with herbs on serum kidney function parameters of the hypercholesterolemic rats

The kidney function parameters of control and blended crackers samples are shown in Table (8). From such that it could be indicated that the uric acid, creatinine and urea nitrogen levels of positive rat group were significantly different from the normal control rat group. However treated group with C_{WFt} showed significant decrease ($p<0.05$) in uric acid, creatinine and urea nitrogen levels compared to negative control. While treated groups with C_{ZP} , C_{CP} and C_{MP} showed nonsignificant different compared to normal group.

In similar studies **Roberts *et al.*, (2006) and Hamidian *et al.*, (2009**) reported that consumption of high cholesterol diets which result in metabolic syndrome marked, hyperlipidemia and associated with

oxidative stress and nitric oxide inactivation by reactive oxygen species (ROS) and diminish NO bioavailability which leading to renal dysfunction, characterizing by high level of creatinine and blood urea nitrogen. Thus the herbs powder exhibited their antihyperuricemia effect. Crackers with herbs powder improve renal function as a result of hydroxycitric acid which reduce oxidative stress and declining lipid profiles and level of oxidized LDL which generally improved kidney function **(Igho *et al.*, 2011).**

Table (7): Effect of crackers samples on lipids profile (mg/dl) and atherogenic index of the hypercholesterolemic rats

Groups	TC	TG	HDL-C	LDL-C	VLDL-C	Atherogenic Index
Negative control	55.74 ± 1.05 ^{d}	64.20 ± 1.74 ^{d}	14.31± 0.16 b ^{c}	28.59	12.84	2.00
Positive control	96.01 ± 1.06 ^{a}	111.94 ± 1.48 ^{a}	18.79± 0.29 ^{a}	57.83	22.39	3.66
Group 3 (C_{wf})	87.69 ± 0.74 ^{b}	98.23 ± 0.97 ^{b}	15.97± 0.12 ^{b}	55.88	17.86	3.51
Group 4 (C_{ZP})	73.44 ± 1.78 ^{c}	85.28 ± 1.07 ^{c}	14.91± 0.16 ^{b}	44.48	15.06	2.25
Group 5 (C_{cp})	74.73 ± 0.70 ^{c}	88.47 ± 0.64 ^{c}	15.92± 0.21 ^{b}	42.11	17.69	2.28
Group 6 (C_{MP})	73.81 ± 1.41 c	86.33 ± 0.45 c	15.74± 0.12 b	41.80	17.27	2.29

Values are expressed as means ± SD. Values with the different letters in the same column are significantly difference at P≤0.05. TC: Total Cholesterol, TG: Triglycerides, HDL-c: High density lipoprotein, LDL-c: Low density lipoprotein, VLDL-c: Very low density lipoprotein.

Table (8): Effect of crackers samples on serum uric acid creatinine and urea of the hypercholesterolemic rats

Groups	Uric acid (mg/dl)	Creatinine (mg/dl)	Urea nitrogen (mg/dl)
Negative control	1.22 ± 0.05 ^{c}	1.25 ± 0.02 ^{b}	15.9 ± 0. 41 ^{c}
Positive control	2.28 ± 0.02 ^{a}	1.41 ± 0.02 ^{a}	24.2 ± 0. 63 ^{a}
Group 3 (C_{wf})	1.73 ± 0.05 ^{b}	1.33 ± 0.01 ^{ab}	20.6 ± 0. 31 ^{b}
Group 4 (C_{ZP})	1.33 ± 0.05 ^{c}	1.29 ± 0.03 ^{b}	18.5 ± 0. 25 ^{c}
Group 5 (C_{cp})	1.28 ± 0.07 ^{c}	1.27 ± 0.03 ^{b}	17.3 ± 0. 11 ^{c}
Group 6 (C_{MP})	1.29 ± 0.05 ^{c}	1.26 ± 0.02 ^{b}	17.9 ± 0. 41 ^{c}

Values are expressed as means ± SD. Values with the different letters in the same column are significantly difference at P≤0.05

Effect of crackers samples on serum liver function parameters of the hypercholesterolemic rats

The kidney function parameters of control and blended crackers samples are shown in Table (9). From such that it could be observed that the positive control showed significant increase in ALT, AST and ALP at $p < 0.05$ in comparing with normal control group. The treated groups C_{ZP}, C_{CP} and C_{MP} had the lowest ALT, AST and ALP levels followed by C_{WFt}

In hypercholesterolemia condition, the liver is shelling by the free fatty acids that flow out of the adipose tissues into the portal blood. This can directly cause inflammation within cells of the liver, which then released more pro-inflammatory cytokines, leading in liver injury and cells (**Fielding and Frayn 2000 and Adeniran *et al.*, 2013).** Ziziphus are concent the presence of tannins, saponins and phenolic compounds observed in could be responsible for the observed effects of decreasing the levels of injure in liver and lipid peroxidation **(Dahiru and Obidoa 2008)**. Also, **Mukherjee *et al.*, (2010)** indicated that curry is the most herbal plant which can protected and mitigate the liver function, which was show by the capacity of this polyphenol to modifiy the antioxidant defense and to reduce the lipid peroxidation in these tissues. Furthermore **Lobna *et al.*, (2014)** the results revealed that the rats treated with marjoram powder and oil showed significant decrease in serum ALT and AST.

Table (9): Effect of crackers samples on serum ALT, AST, and ALP activities (mmol/L) of the hypercholesterolemia rats

Groups	ALT (µ/L)	AST (µ/L)	ALP (µ/L)
Negative control	36.15 ± 1.6^{c}	62.62 ± 1.8^{c}	85.59 ± 1.9^{c}
Positive control	60.57 ± 2.4^{a}	92.45 ± 2.1^{a}	118.41 ± 1.2^{a}
Group 3 (C_{wf})	46.32 ± 2.6^{b}	71.65 ± 2.3^{b}	99.71 ± 2.5^{b}
Group 4 (C_{ZP})	37.78 ± 2.8^{c}	64.83 ± 2.1^{c}	87.92 ± 2.8^{c}
Group 5 (C_{cp})	39.12 ± 2.2^{c}	63.98 ± 2.4^{c}	88.03 ± 2.2^{c}
Group 6 (C_{MP})	38.15 ± 1.6^{c}	63.62 ± 1.8^{c}	87.59 ± 1.9^{c}

Values are expressed as means ± SD. Values with the different letters in the same column are significantly difference at $P \leq 0.05$. AST: Aspartate aminotransferase ALT :Alanine aminotransferase ALP: Alkaline phosphatase

Effect of crackers samples on serum leptin hormone and adiponectin of the hypercholesterolemic rats

The leptin hormone and adiponectin of control and blended crackers samples are shown in Table (10). From such that it could be observed that the significant increase on the leptin levels of positive control comparing with normal control. There is marked increase in the level of adiponetin of treated groups C_{WFt} C_{ZP}, C_{CP} and C_{MP} while decreased in the level of leptin hormone of treated groups C_{WFt} C_{ZP}, C_{CP} and C_{MP} when compared to positive control. In similar studies **Vanderpump and Tunbridge (2000), Middleton *et al.*, (2000) and Goodman and Gilman (2006)** found that high fat diet caused a marked hypertrophy of brown and white adipose tissue. Hyperthyroidism is associated with abnormal lipid profile and high adiponectin. This may be a possible explanation for the high cardiovascular morbidity among hyperthyroidic. It seems that, the effect of fortified crackers with herbs powder on reducing leptin hormone is known to be due to its powerful antioxidant activities.

Table (10): Effect of crackers samples of serum on leptin hormone and adiponectin of the hypercholesterolemia rats

Groups	Leptin (ng/ml)	Adiponetin (U/mL)
Negative control	2.97± 0.07 [d]	8.67±0.06 [a]
Positive control	5.04±0.11 [a]	4.98±0.05 [d]
Group 3 (C_{wf})	4.85±0.05 [b]	6.28±0.09 [c]
Group 4 (C_{ZP})	3.47±0.05 [c]	8.14±0.13 [b]
Group 5 (C_{cp})	3.52±0.02 [c]	8.18±0.14 [b]
Group 6 (C_{MP})	3.66±0.07 [c]	8.25±50.09 [b]

Values are expressed as means ± SD. Values with the different letters in the same column are significantly difference at $P \leq 0.05$

Effect of crackers samples on serum total antioxidant capacity (TAC) superoxide dismutase (SOD) activity, malondialdehyde (MDA) and acetylcholine esterase (AChE) of the hypercholesterolemia rats

The leptin hormone and adiponectin of control and blended crackers samples are shown in Table (10). From such that it could be revealed that levels of SOD and TAC for normal control rats were (28.97±.54a µ /ml and 2.9 ±.58 U/mg), while the corresponding levels for positive control group were lower (1.32±.35 U/mg and 20.54 ±.71 µ /ml).

Data showed a significant increase in SOD and TAC levels and a significant decrease in MDA in all rat groups (Table 11) which treated with fortified herbs cracker as compared to the positive control groups. The treated rat group C_{ZP}, C_{CP} and C_{MP} had the highest acetylcholine esterase (AChE) levels which was similar to the values of normal control group. Meanwhile the lowest TAC, SOD and acetylcholine esterase (AChE) and highest MDA values were for positive control group. Fortification with herbs to diet increased antioxidant parameters such as TAC, SOD, MDA and acetylcholine esterase AChE levels. Research interest has focused on various herbs that possess hypolipidemic, antiplatelet, antitumor, or stimulating properties of immunty may be useful in helping reduce the risk of cardiovascular disease and cancer. In different herbs, a wide variety of active phytochemicals such as phenols and flavonoids may be activity of protective enzymes such as the enzyme glutathione transferase. Natural coloring agents from herbs contain potent antioxidant compounds that provide significant protection against chronic diseases. These compounds may protect LDL cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, inhibit lipid peroxidation, or have antiviral or antitumor activity. This might be linked to strong antioxidant activities, inhibition of α-glucosidase and α-amylase, inhibition of acetylcholine esterase enzymes (ACE) (**Giordano *et al.*, 2007, and Gokcimen *et al.*, 2007)**

Table (11): Effect of crackers samples on proximate serum total antioxidant capacity (TAC) superoxide dismutase (SOD) activity, malondialdehyde (MDA) and acetylcholine esterase (AChE) of the hypercholesterolemia rats

Groups	TAC (U/mg)	SOD (μ /ml)	MDA μmol/mg protein	AChE nmol
Negative control	2.99±.58 [a]	28.97±.54 [a]	1.54±.54 [d]	6.19± 0.4 [a]
Positive control	1.32±.35 [d]	20.54±.71 [d]	3.79±.65 [a]	3.54± 0.4 [d]
Group 3 (C_{wf})	2.32±.77 [c]	24.52±.28 [c]	2.11±.58 [bc]	4.96± 0.7 [c]
Group 4 (C_{ZP})	2.85±.68 [b]	26.98±.84a [b]	1.63±.35 [d]	5.85± 0.6 b
Group 5 (C_{cp})	2.84±.66 [b]	26.85±.25 [b]	2.68±.32 [b]	5.89± 0.4 [b]
Group 6 (C_{MP})	2.87±.98 [b]	26.87±.24 [b]	1.64±.74 [d]	5.92± 0.4 [b]

Values are expressed as means ± SD. Values with the different letters in the same column are significantly difference at P≤0.05

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خفض ارتفاع الكوليستيرول فى دم الفئران التى تم تغذيتها على المقرمشات المخلوطة ببعض الأعشاب كمواد تلوين طبيعية

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تهدف هذه الدراسة إلى تقييم تأثير اضافة بعض الأعشاب كمواد ملونة الى المقرمشات لخفض كولسترول الدم فى الفئران. تم استخدام بعض الأعشاب مثل العناب والكاري والبردقوش بنسبة ١٠% لتحسين اللون ورفع القيمة الغذائية للمقرمشات. وقد تم تأكيد النتائج المتحصل عليها من الاختبارات (الريولوجية والكيميائية) من خلال اجراء اختبارات بيوكيميائية. وتم إجراء التجارب البيولوجية باستخدام ٣٦ فأر من الذكور البالغة، قسمت ستة مجموعات (٦ فأر لكل مجموعة) . المجموعة (١) مجموعة كنترول سالبة والتى تغذت على الوجبة القياسية ، أما المجموعات الخمسة الباقيين تغذت على الوجبة القياسية المضاف اليها ٢٪ من الكولسترول وكذلك الحقن فى العضل ب ٠٫٥و٠ مل حامض الكوليك لمدة ٣٠ يوما للحث على ارتفاع كوليسترول الدم. وابقيت المجموعة (٢) كمجموعة موجبة أما المجموعات (٣ و ٤ و ٥ و ٦) فقط تناولت المقرمشات الطبيعية (١٠٠٪ دقيق القمح)، والمخلوطة ب ١٠٪ العناب ، و ١٠٪ الكاري، و ١٠٪ المردقوش. وأوضحت النتائج أن اضاف الاعشاب للمقرمشات أدى الى حدوث انخفاضا ملحوظا فى ترسيب الدهون، صورة دهون الدم (الكوليسترول الكلي، الجليسريدات الكلية، ومستويات الكوليسترول منخفض الكثافة ، ومؤشر تصلب الشرايين)، حامض اليوريك ، والكرياتينين، واليوريا ، و انزيمات الكبد ALT،و AST، وALP، اللبتين و المالونالدهيدات (مؤشر أكسدة الدهون). كما أظهرت النتائج ارتفاعا ملحوظا في مستويات السيرم لكلا من مستويات الكوليسترول عالية الكثافة، وهرمون اديبونيكتين (adiponetin)، ودرجة النشاط المضاد للأكسدة، وأنزيمات SOD، واستريز أستيل عند المقارنة بالمجموعة الضابطة الموجبة والسالبة. لذا توصي الدراسة بتدعيم المقرمشات بالأعشاب الملونة بالعناب، والكاري، والبردقوش والتى يمكن أن تساهم كعلاج ارتفاع كوليستيرول الدم بالاضافة الى فوائد صحية أخرى.

الكلمات المفتاحية: العناب، الكاري، البردقوش، النشاط المضاد للأكسدة، الخواص الريولوجية، الخواص الحسية.