Effect of Mixing Different Types of Spices on Biochemical Parameters and Body Weight Gain in Obese Rats.

AlShimaa Abdalla Hafez, Reda A. M. Hathout

Abstract

Appropriate lifestyle pattern and behavior interventions are crucial to weight loss success but maintaining is extremely challenging. The current study aimed to evaluate the effect of mixing some spices (cinnamon, cardamom, turmeric, ginger, and clove) on biochemical parameters and body weight gain in obese rats. This work was carried out on (40) Adult male Wister albino rats (weighing 120 ± 20 g) randomly classified into five groups. control (c-), group (2) was fed with High Fat Diet (c+). group (3,4, and 5) fed with HFD + different mixing of spices (cinnamon, cardamom, turmeric, ginger, and clove) throughout the experimental period of 8 weeks. Chemical composition, Mineral's content, fatty acid, phenolic compounds, biochemical signs, Seram glucose level, body weight gain, liver & kidney function and histopathological examinations were determined. The results showed that there was a significant difference in Chemical composition and fatty acids content between the three tested samples. according to Biochemical Parameters treated groups with the three mixes shows a significant decrease in Cholesterol, Triglyceride, and LDL-c, and a significant increase in HDL-c. especially the group fed on mix 1 reported a great improvement in Cholesterol, Triglyceride, HDL-c, and LDL-c, compared with groups fed on mix 2 or mix 3. the three treated groups showed a significant improve in Seram glucose level. and a significant decrease in (BWG) especially the group fed on mix 3.

it could be noticed that the mixing several medical spices together will provides more significant effect in treating obesity, hyperlipidemia, and high blood glucose levels especially mix (1) was the most effective mix.

Keywords: obesity, Spices, Biochemical Parameters, cinnamon, ginger, cardamom, Turmeric, clove, rats.

Introduction

Obesity is a great public health problem whose prevalence has been rapidly increasing in the United States (U.S), and

globally. It is one of the important causes of preventable deaths globally and contributes to the development of many diseases ***1.*** Obesity results from the long-term or cumulative excessive consumption of energy that is expended by the body. It is also a chronic disease that involves genetics, metabolic, hormonal, and behavioral components. The prevalence of obesity is increasing around the world, particularly in developed countries. Worldwide, in 2016 more than 340 million children and adolescents aged 5-19 were overweight or obese which estimated to be 70 million by 2025. The highest percentage of overweight or obese children live in developing countries, where the rate of increase has been more than 30% higher than that of developed countries ***2,3.***

Obesity is strongly associated with significant health and economic status. Addition to it is associated with depression, diabetes, high blood pressure, high cholesterol levels, cancers, respiratory disorders in sleep, osteoarthritis, gallbladder disease, asthma, arthritis, pregnancy complications, menstrual disorders, urinary stress incontinence, low quality of life, discrimination, and early mortality ***4.***

The main therapeutic options to treat obesity include diet modification, exercise, surgery, and pharmacotherapy. concerning conventional weight loss therapies often do not have satisfactory outcomes, and the prevalence of obesity is predicted to continue to increase ***5.*** A lot of dietary plants have shown anti-obesity activity by reducing appetite, inhibiting nutrient absorption, reducing adipogenesis, and enhancing energy expenditure. In recent years, the influence of gut microbiota on obesity has become a great research focus and is a potentially effective target to treat obesity. The impact of dietary plants on obesity has become a particular focus of attention ***6.***

Spices are the dried parts of the plants. The great difference between an herb and a spice is that spice comes from any part of a plant other than the leaves while an herb always comes from the leaves ***7.*** Spices typically come from the dried part of a plant such as buds, flowers (cloves, saffron); bark (cinnamon); root (ginger,

turmeric); fruits/berries (cloves, chili, black pepper); or seeds (cumin) that contain volatile oils or aromatic scents and flavors ***8.*** Spices are among the most used plant species either for their nutritional or medicinal purposes. Nutritionally, spices are dietary adjuncts primarily used as flavoring agents to garnish the taste of foods. Also, they are essential medicines that have been used for millennia ***9.*** Spices not only enhance the flavor, aroma, and color of food and beverages but can also protect them from acute and chronic diseases.

Herbs and spices are rich sources of bioactive phytochemicals, and the greatest component of these phytochemicals has powerful antioxidant activity. Phenolic compounds (phenolic acids, flavonoids, terpenes etc.) are the main phytochemicals that are responsible for the antioxidant activity in herbs and spices ***10.***

The antioxidant properties of herbs and spices have been presented as a wide range of bioactive compounds in herbs and spices have been studied to reveal their antioxidant capacity ***11.*** Dietary phytochemicals might be employed as anti-obesity agents because they may suppress the growth of adipose tissue, inhibit differentiation of preadipocytes, stimulate lipolysis, and induce apoptosis of existing adipocytes, thereby reducing adipose tissue mass ***12.***

Nowadays conventional therapies such as restricted diet and changing lifestyle have not come out so efficacious. people are desperate for new, easier, and more successful methods such as supplementation with herbal remedies to reach their ideal body shape ***13.***

Turmeric is generally used in meals and gives flavor and yellow color to curry. In addition to making mustard sauce, it is also used for coloring butter and cheese. Turmeric is preferred as an anti-inflammatory agent in traditional medicine for the treatment of skin disorders, wounds, digestive, and liver problems ***14.*** curcumin is the main pigment of turmeric. Curcumin, together

with other related pigments, gives the rhizome of the plant a yellow color. Chemically, these pigments are polyphenols, which are called curcuminoids. Turmeric and especially curcumin have been demonstrated in detail for their antioxidant and anti-inflammatory properties in recent years and the results showed that curcumin is a potent antioxidant and immunomodulatory ***15.***

Cinnamon is a flavor additive used for improving the odor, taste, and color of meals for a long time. It is derived from the inner bark of several tree species from the genus Cinnamomum widely found in the Mediterranean region, Sri Lanka, and India. ***16.*** Cinnamon is also high in antioxidants such as polyphenols and glutathione therefore, it could be presented as a powerful anti-inflammatory agent and may protect against cancer ***17***. Also, it could be lowered the risk of heart diseases by reducing LDL cholesterol and increasing HDL cholesterol ***18.***

Ginger is used for culinary purposes. It contains various phytochemicals and biologically active components, such as gingerols and shogaols. Apart from other traditional medical uses, ginger and its main bioactive constituents were reported to attenuate obesity both in rodent animal models and cell lines ***19.*** The main pharmacological actions of ginger and its isolated compounds include immunomodulatory, anticancer, anti-inflammatory, anti-apoptosis, glucose, lipid lowering effect and antiemetic ***20.*** Ginger has been shown to possess antidiabetic activity in a variety of studies. Other studies suggested that the response to ginger components depends on its dose concentration ***21.***

Clove is a spice name representing a small reddish-brown dried flower bud of Syzygies aromatical. clove is among the first group of spices known to be traded by humans. The primary use of clove is for flavoring and seasoning or other culinary uses ***22.*** The essential oils of primarily eugenic composition have been traditionally used for treating many diseases. The preservative nature of the oils associated with antimicrobial activity has also been a significant feature in food preservation and traditional

medicine. Treating bad breath and dental care with clove has been reported throughout the history of traditional uses of clove by mankind. In fact, the essential oil has an antibacterial effect against those strains responsible for dental caries and periodontal diseases ***23.***

Cardamom is used in the form of whole fruit, decorticated seeds or ground seeds. Cardamon plays an important role in both sweet and savory cuisine worldwide. It is widely used in many Indian sweetmeats, drinks, and desserts such as the popular ice cream. In Arabic countries, it is widely and popularly used to flavor coffee and tea and cardamom powder is used as a spice for sweet dishes ***24.*** Cardamom is added a little to ground coffee before brewing, then sweeten and top with cream. In Turkey, it is used to flavor the black Turkish tea called Kakakule. Cardamom oil is an important ingredient in food preparations and health foods. Cardamom is listed in the medical pharmacopeias of several Asian countries. anti-inflammatory, diuretic, abortifacient, analgesic, desiccant, resolvent and has been used asthma, constipation, colic, diarrhea, dyspepsia, hypertension, epilepsy, bronchitis, piles, consumption, strangury, scabies, pruritus, bladder and kidney diseases, lung congestion ***25.***

The current study aimed to evaluate the effect of mixing some spices (cinnamon, cardamom, turmeric, ginger, and clove) on biochemical parameters and body weight gain in obese rats.

Materials and Methods

Samples:

The spices powder (cinnamon, ginger, cardamom, Turmeric, and clove) was obtained from Haraz company for medical herbs, Cairo, Egypt. three mixes of spices powder have been made: mix (1) consist of (5g/kg b.wt cinnamon + 5g/kg b.wt ginger + 3g/kg b.wt cardamom). mix (2) consist of (5g/kg b.wt Turmeric + 5g/kg b.wt ginger + 3g/kg b.wt clove). mix (3) consist of (5g/kg b.wt cinnamon+ 5g/kg b.wt ginger + 3g/kg b.wt cardamom+5g/kg b.wt Turmeric + 3g/kg b.wt clove***). 26, 27,28, 29,***

Chemical composition of samples:

Moisture, protein, fat, fiber, and ash contents of samples were determined according to the methods of **A.O.A.C (2005)** ***30.*** Total carbohydrates were calculated by the differences. All proximate composition experiments were performed in triplicate and expressed as g/100 g of samples on dry basis Mineral contents (calcium, potassium, sodium, iron, and zinc) were determined using a Pye Unicum SP1900 Atomic Absorption Spectroscopy instrument as described by **A.O.A.C (2005)** ***30.***

The fatty acids content of samples:

Methylation of fatty acids:

An aliquot of fatty acids after saponification and acidification of samples, about 10 mg, was dissolved in 2ml hexane and then 0.4 ml of 2N KOH in anhydrous methanol was added ***31,*** after 3 min, 3 ml water was added. The organic layer, separated by centrifugation, was dried over anhydrous sodium sulfate, and then concentrated, with a N2 stream to around 0.5 ml for GC analysis of fatty acids methyl esters (FAME) as described below.

GC analysis of FAME:

Agilent 6890 series GC apparatus provided with a DB-23 column (60 m×0.32 mm× 0.25 µm) was used. Oven temperatures were 150°C ramped to 195°C at 5°C min -1, ramped to 220°C at 10°C min -1 and flow rate was 1.5min -1. Fatty acids result after the steps of the previous procedures were transformed into methyl esters and directly injected into the GC ***32.***

Determination of phenolic compounds:

Phenolic compounds of all mixtures of spices (Mix1, Mix2, and Mix3) powder were determined by HPLC according to the method of (Agilent ,2014) in faculty of Agriculture, food safety and control lab., Cairo University. Agilent 1260 infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, akinetex® 5µm EVO C18 100×4.6mm, (Phenomenex, USA),

operated at 30ºc. The separation is achieved using a ternary liner elution gradient with (A) HPLC grade water 0.2% H3PO4 (v/v), (B) methanol and (C) acetonitrile. The injected volume was 20µL. Detection: VWD detector set at 284 nm.

Animals. (40) Adult male Wister albino rats (weighing 120 ± 20 g) were obtained from the animal house, Agricultural research center, Cairo University, Egypt. Animals were kept in wire bottomed cages, in a room under a standard condition of illumination with a 12 h light-dark cycle at 25 ±1°C. They were provided with water and a balanced diet ad libitum.

Experimental Diets and Design. After acclimatization for 2 weeks, the animals were randomly divided into two main groups, (1) normal control group 8 rats (was received a regular diet throughout the experimental period of 16 weeks). (2) The experimental group (32 rats) for obesity induction, animals were fed with High Fat Diet for 8 weeks and compared with rats fed a normal diet (The composition of diets (control and HFD) is shown in Table (1).

then divided into four subgroups 8 rats in each, one control positive group and three treatment groups.

control positive group fed with HFD only throughout the experimental period of 8 weeks, (I) mix 1 group fed with HFD containing (5g cinnamon+ 5g ginger + 3 g cardamom /kg b.wt.) for 8 weeks, (II) mix 2 group fed with HFD containing (5g Turmeric + 5g ginger + 3 g clove /kg b.wt.) for 8 weeks, and (III) mix 3 group fed with HFD containing (5g cinnamon+ 5g ginger + 3 g cardamom+5g Turmeric + 3 g clove /kg b.wt.) for 8 weeks.

Supplemented diets were prepared by combining HFD meals with the three different mixes. Diets were stored in airtight containers at 4 0C in a refrigerator.

Body weights were measured weekly for all groups, and every other week, blood was collected for blood glucose analysis. At the end of the study, blood was also collected by cardiac puncture after an overnight fast for the determination of plasma

lipid profile levels, liver, and kidney function. and all rats were sacrificed by cervical dislocation and liver, kidney and heart samples were removed and weighed then stored in formalin 10% until analyses.

Table 1: Composition of diets (g/kg diet) fed to rats.

	Control	HFD
Casein	200.0	200.0
Starch	579.5	150.0
Sucrose	50.0	149.5
Soybean oil	70.0	—
Beef tallow	—	400.0
Cellulose	50.0	50.0
Vitamin-mineral premix∗	45.0	45.0
l-Cysteine	3.0	3.0
Choline bitartrate	2.5	2.5

∗The vitamin-mineral premix provides the following (per kg): all-*trans*retinyl acetate, 1.8mg; cholecalciferol, 0.025mg; all-*rac*-a-tocopherol acetate, 12.5mg;menadione (menadione sodiumbisulfate), 1.1mg; riboflavin, 4.4mg; thiamine (thiaminemononitrate), 1.1mg; vitamin B-6, 2.2mg; niacin, 35mg; Ca-pantothenate, 10 mg; vitamin B-12, 0.02mg; folic acid, 0.55 mg; *d*-biotin, 0.1mg; manganese (from manganese oxide), 40mg; iron (from iron sulfate), 12.5mg; zinc (from zinc oxide), 25mg; copper (from copper sulfate), 3.5mg; iodine (from potassium iodide), 0.3mg; selenium (from sodium selenite), 0.15mg; choline chloride, 175mg. ***33***

Blood sampling and biochemical assays:

Blood was collected, centrifuged, and plasma was used immediately for glucose level measurement. Serum triglyceride (TG) was determined according to the method of *(Koditscheck & Umdreit, 1969)* ***34.*** serum total cholesterol (TC) was determined according to the method of *(Richmond, 1973)* ***35.*** Serum LDL-cholesterol was determined according to the method of *(Levy, 1981)* ***36***, while Serum HDL- cholesterol was determined according to the method of *(Burstein, 1970)* ***37.***

Liver function test:

Colorimetric determination of alanine aminotransferase (GOT) or aspartate aminotransferase (GPT) was estimated by

measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4-dinitrophenylhydrazine, according to the method of *Reitman and Frankel (1957)* ***38.***

Kidney function test:

Uria, and serum creatinine (Cr) were assayed in serum, using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the methods that were described by *Fossati et al. (1980)* ***39****, Fawcett and Scott (1960)* ***40*** *and Szasz et al. (1979)* ***41*****,** respectively**.**

Histopathologic Examinations:

Liver, kidneys, and heart specimens from all groups (3 rats from each group) at the last week of the experiment were collected and fixed in neutral buffered formalin 10%, routinely processed and embedded in paraffin wax. Paraffin blocks were sectioned at 4-5 µm thickness and stained with Hematoxylin and Eosin for histopathological examination by light microscope (Olympus BX50, Japan). ***42***

Statistical Analysis: Data were stated as mean ±SD. The alteration among groups was analyzed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test (SAS Institute: SAS User's Guide: Statistics). and $P < 0.05$ was considered statistically significant.

Results

Chemical composition of the tested samples:

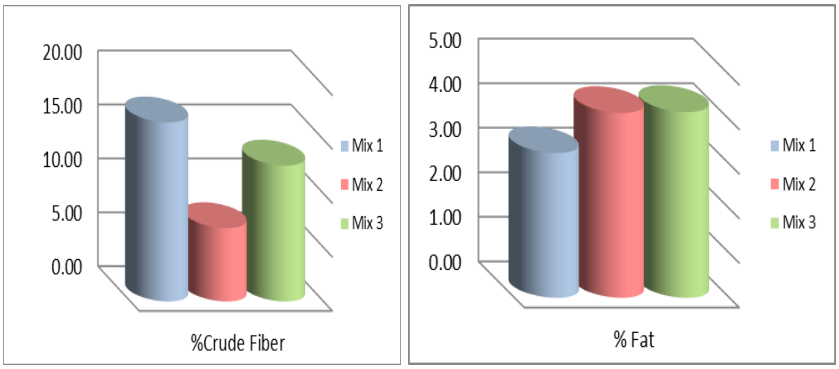
The content of protein, fat, ash, crude fiber, moisture, and carbohydrate of the tested samples are shown in Table (2) and figure (1). as seen in the table (2) and figure (1). there was a significant difference in Chemical composition between the tested samples**, mix 1** was the highest content of crude fiber (16.60%), compared with mix 2 (6.80%) and mix 3 (12.60%). while it was the lowest content of protein (6%), fat (3.25%), ach (4.60%), and moister (10.30%) compared with mix 2 (7.60%, 4.15%, 5.80%,

and 12.80% respectively), and mix 3 (7.10%, 4.17%, 5.20%, and 12.00% respectively).

table (2): Chemical composition of the tested samples (g/100g dry sample).						
	Protein %	**% Fat**	**%Ach**	**%Crude Fiber**	**% Moister**	**T. C ****
Mix 1	6.00 b ± 0.63	3.25 b ± 0.37	4.60 b ± 0.38	16.60 a ± 0.45	10.30 c ± 0.58	59.25 b ± 1.93
Mix 2	7.60 a ± 0.44	4.15 a ± 0.33	5.80 a ± 0.65	6.80 c ± 0.64	12.80 a ± 0.42	62.85 a ± 2.34
Mix 3	7.10 a ± 0.32	4.17 a ± 0.13	5.20 ab ± 0.60	12.60 b ± 0.55	12.00 b ± 0.76	58.93 b ± 2.94
F	17.506	18.621	6.953	479.105	26.673	4.787
Sig.	0.000	0.000	0.007	0.000	0.000	0.025

T.C** = Total carbohydrates calculated by difference

figure (1) Chemical composition of the tested samples

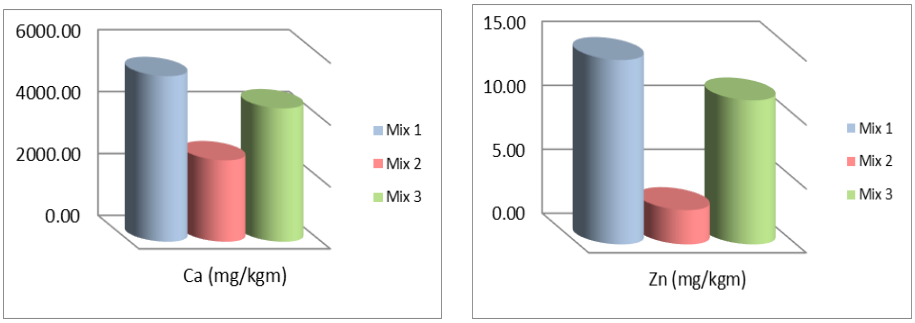


Mineral's content:

The content of calcium, potassium, sodium, iron, and zinc in the tested samples are shown in Table (3) and figure (2)

Table (3): Mineral's content of the tested samples:					
	Ca (mg/kg)	**K %**	**Na (mg/kg)**	**Fe (mg/kg)**	**Zn (mg/kg)**
Mix 1	5,353.00 a ± 157.55	1.84 c ± 0.01	306.20 c ± 3.35	142.90 b ± 3.73	14.43 a ± 0.68
Mix 2	2,633.00 c ± 90.94	2.53 a ± 0.14	1,260.00 a ± 31.22	287.30 a ± 7.50	2.69 c ± 0.13
Mix 3	4,312.00 b ± 65.92	2.15 b ± 0.06	701.30 b ± 4.74	290.20 a ± 2.17	11.27 b ± 0.32
F	905.616	88.688	4,100.834	1,703.951	1,135.381
Sig.	0.000	0.000	0.000	0.000	0.000

figure (2): Mineral's content of the tested samples

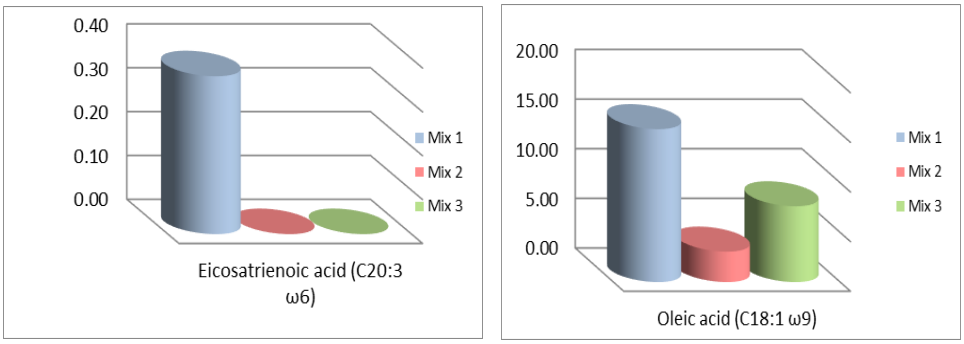


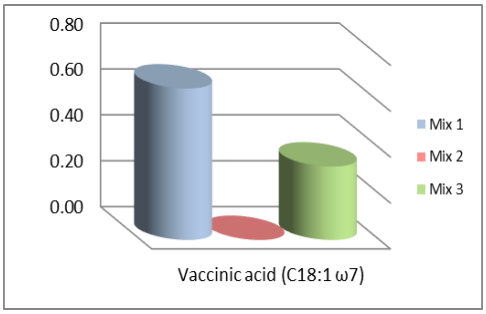
as showed in Table (3) and figure (2) there was a significant difference in Mineral's content between the tested samples**, mix 1** was the highest contain of Calcium Ca (5.353mg/kg.) and Zink Zn (14.43 mg/kg.), compared with **mix 2** (2.633 mg/kg., and 2.69 mg/kg respectively) and **mix 3**(4.312 mg/kg, and 11.27 mg/kg respectively). while **mix 2** was the highest contain of potassium K (2.53 %) and Sodium Na (1.260.00 mg/kg.), compared with **mix3**(2.15%, and 701.30 mg/kg. respectively) and **mix 1** (1.84%, and 306.20 mg/kg. respectively) Whereas the contain of Iron Fe was the highest in mix3 (290.20 mg/kg) compared with mix 2 (287.30 mg/kg) and mix 1 (142.90 mg/kg) respectively.

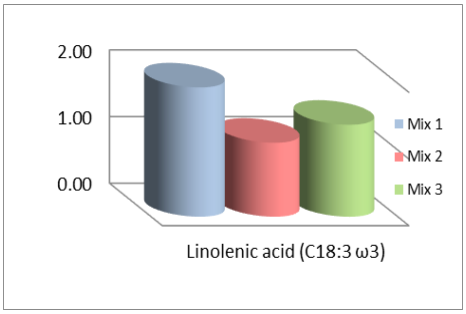
The fatty acids content:

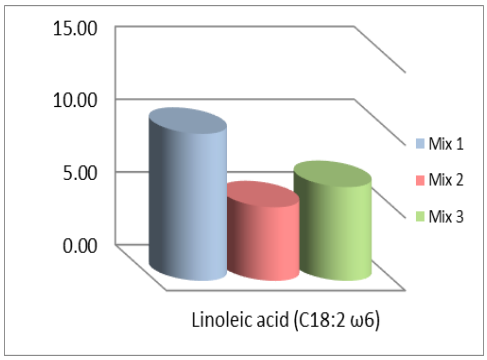
Table (4) and figure (3) show the fatty acids content of the tested sample

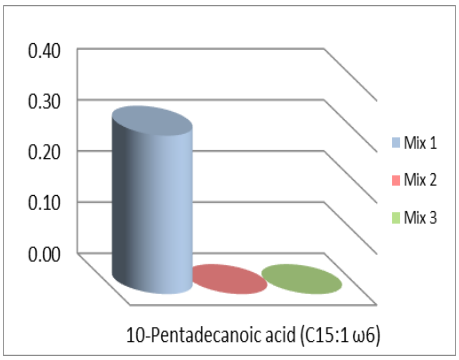
figure (3): the fatty acids content of the tested samples

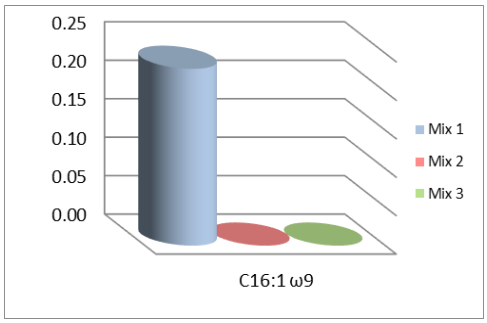












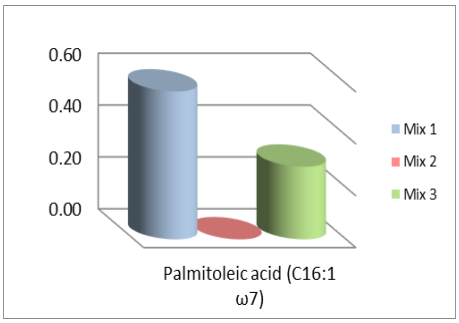


	Table (4) the fatty acids content of the tested samples				F	Sig.
	Fatty acid	Mix1	Mix2	Mix3		
1	Caproic acid(C6:0)	1.61 a ± 0.36	0.00 ± 0.00	0.91 b ± 0.42	37.516	0.000
2	Caprlyic acid (C8:0)	6.57 a ± 0.62	1.46 c ± 0.34	4.58 b ± 0.58	141.519	0.000
3	Caprie acid (C10:0)	1.52 a ± 0.30	1.07 b ± 0.17	0.88 b ± 0.08	15.468	0.000
4	U0ecanoic acid (C11:0)	16.26 b ± 0.65	18.77 a ± 0.55	13.03 c ±0.87	101.182	0.000
5	Behenic acid (C22:0)	0.29 a ± 0.06	0.15 b ± 0.04	0.18 b ± 0.04	13.288	0.000
6	Non identified fatty acids	0.34 b ± 0.06	0.55 a ± 0.08	0.00 ± 0.00	133.327	0.000
7	Vaccinic acid (C18:1 ω7)	0.66 a ± 0.06	0.00 ± 0.00	0.32 b ± 0.06	269.341	0.000
8	Linoleic acid (C18:2 ω6)	10.11a ± 0.69	5.06 c ± 0.48	6.45 b ± 0.78	92.951	0.000
9	Linolenic acid (C18:3 ω3)	1.94 a ± 0.51	1.11 b ± 0.02	1.38 b ± 0.15	11.236	0.000

10	**Arachidic acid (C20:0)**	0.42 a ± 0.06	0.19 b ± 0.03	0.23 b ± 0.07	28.671	0.000
11	**Eicosatrienoic acid (C20:3 ω6)**	0.36 a ± 0.04	0.00 ± 0.00	0.00 ± 0.00	452.093	0.000
12	**C16:2 ω4**	1.08 a ± 0.11	0.20 c ± 0.06	0.34 b ± 0.05	222.611	0.000
13	**Hexagonic acid (C16:3 ω4)**	0.00 ± 0.00	0.38 a ± 0.04	0.26 b ± 0.04	223.421	0.000
14	**C16:4 ω3**	0.00 ± 0.00	0.26 b ± 0.02	0.51 a ± 0.12	75.425	0.000
15	**Stearic acid C18:0)**	1.78 c ± 0.26	4.59 a ± 0.47	3.85 b ± 0.60	58.729	0.000
16	**Oleic acid (C18:1 ω9)**	15.41 a ± 0.65	3.07 c ± 0.63	7.64 b ± 0.54	630.376	0.000
17	**10-Pentadecanoic acid (C15:1 ω6)**	0.31 a ± 0.06	0.00 ± 0.00	0.00 ± 0.00	169.588	0.000
18	**Palmitic acid (C16:0)**	11.98 c ± 0.50	33.48 a ± 4.73	28.46 b±1.63	90.197	0.000
19	**C16:1 ω9**	0.23 a ± 0.06	0.00 ± 0.00	0.00 ± 0.00	93.353	0.000
20	**Palmitoleic acid (C16:1 ω7)**	0.57 a ± 0.07	0.00 ± 0.00	0.28 b ± 0.04	234.327	0.000
21	**Heptadecanoic acid (C17:0)**	1.53 c ± 0.31	7.28 a ± 0.66	5.63 b ± 0.88	121.381	0.000
22	**Lauric acid (C12:0)**	11.10 a ± 0.29	5.67 c ± 0.83	9.39 b ± 0.77	102.438	0.000
23	**Tridecanoic acid (C13:0)**	4.20 c ± 0.60	9.63 a ± 0.93	8.22 b ± 0.65	86.615	0.000
24	**Myristic acid (C14:0)**	6.19 a ± 0.76	4.95 b ± 0.23	4.45 b ± 0.43	17.831	0.000
25	**C14:1 ω9**	2.12 a ± 0.26	1.34 b ± 0.29	1.55 b ± 0.31	12.011	0.001
26	**Pentadecanoic acid (C15:0)**	3.41 a ± 0.55	0.79 c ± 0.07	1.49 b ± 0.40	71.936	0.000

** Each value represents the mean ± SD (standard deviation); statistically significance compared with control group. The alphabet, a,b,c were the statistical difference between treatment groups and control group. a: $P < 0.05$; b: $P < 0.01$; c: $P < 0.001$.

** Each value represents the mean ± SD (standard deviation); statistically significance compared with control group. The alphabet, a,b,c were the statistical difference between treatment groups and control group. a: $P < 0.05$; b: $P < 0.01$; c: $P < 0.001$

as seen in the table (4) and figure(3) there was a significant difference in fatty acids content between the three tested samples, **mix 1** was reported a highly contain of monounsaturated fatty acids (omega 9 fatty acids) such as Oleic acid (C18:1 ω9), C16:1 ω9, C14:1 ω9 and polyunsaturated fatty acids (omega 6 and omega 3 fatty acids) such as Linoleic acid (ω6), Linolenic acid (ω3), 10- Pentadecanoic acid (ω6), and Eicosatrienoic acid (C20:3 ω6). also, a highly contain (omega 7 fatty acids) such as Vaccinic acid (C18:1 ω7) and Palmitoleic acid (C16:1 ω7), whereas has a low content of saturated fatty acids such as Stearic acid C18:0), Palmitic acid (C16:0), Heptadecanoic acid (C17:0), Tridecanoic acid(C13:0), compared with the fatty acids contain in mix 2 or mix 3.

Phenolic compounds

Table (5) and fig. (4,5, and 6) show The Phenolic compounds of the tested samples powder mg/kg. It was observed that the highest percentage of phenolic compound in Mix (1) was salyclic acid (349.60), catechein (221), ellagic acid (176.60), rosemarinic (106.10), rutin (83.80), ferulic acid (12.30), o.coumaric acid (8.18) and pyrogallo (5.02). while the highest percentage in Mix (2) were quinol (257.03), myricetin (106.7), vanillin (23.20) and catechol (20.70). The highest percentage of Mix (3) were neringein (272.2), p-hydroxibenzoic acid (107.10), vanalic acid (89.8), Benzoic acid (73.9), caffeine (48.05) and gallic acid (12.90).

table (5) The Phenolic compounds of the tested samples powder mg/kg

	Phenolic compound	Mix1	Mix2	Mix3
1	Benzoic acid	1830.6	3240.5	73.9
2	Salycilic acid	349.6	45.2	63.3
3	Neringein	238.7	267.5	272.2
4	Catechein	221	105.3	154
5	Ellagic acid	176.6	17.5	30.7
6	rosemarinic	106.1	81.7	95.2
7	Rutin	83.8	32.4	52.03
8	p-hydroxy benzoic acid	59.8	103.3	107.1
9	Myricetin	59.3	106.7	98.8
10	Vanillic acid	32.8	45.4	89.8
11	Kampherol	27.06	30.9	32.2
12	Chlorogenic acid	16.7	36.9	40.3
13	Catechol	12.7	20.7	-------
14	Ferulic acid	12.3	4.7	8.1
15	o-coumaric acid	8.18	6.7	7.1
16	Quinol	6.6	257.03	-------
17	Syringic acid	5.9	17.5	18.4
18	Pyrogallo	5.02	3.3	-------
19	Gallic acid	4.29	12.4	12.9
20	Caffeic acid	4.19	6.07	6.6
21	vanillin acid	1.4	23.2	3.2
22	Caffeine	--------	47.14	48.05
23	Quercitin	---------	61.1	65.4
24	P-coumaric acid	--------	12.8	14.6
25	Cinnamic acid	----------	1.01	1.03

Fig.4 Mix (1)

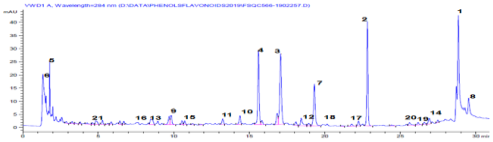


Fig.5 Mix (2)

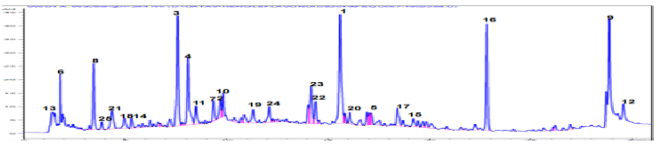
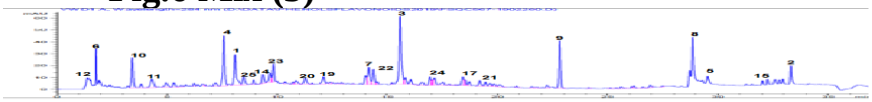


Fig.6 Mix (3)



Effect of the three mixes on Biochemical Parameters:

As shown in table (6) Cholesterol, Triglyceride, and LDL-c increased greatly after feeding on HFD (C+) compared with (C-), while HDL-c was decreased. on another side groups treated with the three mixes shows a significant decrease in Cholesterol, Triglyceride, and LDL-c, and a significant increase in HDL-c. especially the group fed on mix 1 and mix 2 reported a great improvement in Cholesterol, Triglyceride, HDL-c, and LDL-c compared with groups fed on mix 3.

Table (6): the effect of the three mixes on biochemical parameters (n = 8 rats for each group). mg/dl

	Cholesterol	Triglyceride	HDL-c	LDL-c
C-	74.88 c ± 0.93	81.07 b ± 14.29	17.67 d ± 0.72	41.00 a ± 1.30
C+	129.91 a ± 4.86	154.90 a ± 4.73	15.53 a ± 1.60	83.40 c ± 5.38
Mix1 group	74.35 c ± 5.55	81.27 b ± 4.68	25.23 bc ± 2.25	32.87 b ± 2.57
Mix2 group	81.98 bc ± 5.87	84.53 b ± 5.94	19.70 b ± 2.33	47.47 b ± 2.55
Mix3 group	87.30 b ± 18.14	83.73 b ± 4.25	22.43 ± 3.62	48.13 ± 13.89
F	38.27	104.00	16.53	47.51
Sig.	0.00	0.00	0.00	0.00

Effect of the three mixes on serum glucose levels

Table (7): the effect of the three mixes on serum glucose (n = 8 rats for each group).	
	Glucose mg/dl
C-	99.47 b ± 4.80
C+	156.67 a ± 58.25
Mix1 group	93.20 b ± 4.68
Mix2 group	85.90 b ± 1.73
Mix3 group	91.97 b ± 2.14
F	7.34
Sig.	0.00

Table (7) show the effect of the three mixes on serum glucose levels of rats.

As shown in table (7) serum Glucose increased greatly after feeding on HFD (C+) compared with (C-), on another side groups treated with the three mixes shows a significant decrease in serum Glucose compared with HFD (C+) group. but it was non-significant changes between the groups treated with the three mixes.

Effect of the three mixes on body weight gain (BWG)

Table (8) show the effect of the three mixes on body weight gain in rats.

Table (8): the effect of the three mixes on body weight gain (BWG) (n = 8 rats for each group)	
Groups	**BWG (g)**
C-	56.38 b ± 3.89
C+	88.13 a ± 4.55
Mix1 group	-30.50 d ± 2.45
Mix2 group	-12.50 c ± 1.69
Mix3 group	-36.63 e ± 2.97
F	2341.33
Sig.	0.00

as shown in table (8) (BWG) increased dramatically after feeding on HFD (C+) compared with (C-), while groups treated with the three mixes shows a significant decrease in (BWG). Especially the group fed on mix 3 reported a great decrease (-36.63) compared with groups fed on mix 1 or mix 2.

Effect of the three mixes on Liver & kidney function

Results are given in Table (9) concerning serum of GPT there are no significant difference between three treated groups compared with positive group. The results of the same table indicated that the serum of GOT significantly increased in Mix (3 and 2) (56.6 U/L and 49.67 U/L) compared with positive group 43.00 U/L. while the data of Mix (1) shown no significant differences compared with the positive and negative groups.

Table (9) Liver and kidney function of rats. (n = 8 rats for each group

Parameters Groups	GPT (U/L)	GOT (U/L)	Urea mg/dl	Creatinine mg/dl
C-	12.33 a ± 0.52	43.67 b ± 10.33	57.40 a ± 14.09	0.97 c ± 0.05
C+	12.00 a ± 0.89	43.00 b ± 4.10	52.27 ab ± 3.46	0.99 bc ± 0.04
Mix1	11.67 a ± 1.37	42.67 b ± 3.61	44.77 b ± 7.98	1.23 a ± 0.25
Mix2	11.67 a ± 1.37	49.67 ab ± 6.71	58.50 a ± 2.27	1.19 ab ± 0.19
Mix3	12.00 a ± 0.00	56.67 a ± 4.41	46.13 b ± 8.70	1.16 abc ± 0.15
F	0.49	5.46	3.34	3.30
Sig.	0.75	0.00	0.03	0.03

The data were presented as mean ± S.D. According to "Duncan" the mean values were arranged in a descending order from "a": "d"

In the same table it was clear that there was significant difference between Mix (1and 3) compared with positive control in the value of urea (44.77, 46.13 and 52.27 mg/dL) respectively while the results of Mix (2) shown significant increase compared with positive group (58.50 and 52.27 mg/dL). On the other hand, the serum of creatinine was significantly increased in Mix (1) compared with positive group (1.23 and 0.99 mg/dL). But there was no significant difference between Mix (2 and 3) compared with positive group.

Relative weight of organs	Relative weights of liver	Relative weights of kidney	Relative weights of heart
C-	7.53 a ± 1.14	1.35 a ± 0.10	0.76 ab ± 0.06
C+	7.82 a ± 0.52	1.33 a ± 0.12	0.82 a ± 0.05
Mix1	6.25 b ± 0.60	1.35 a ± 0.12	0.71 b ± 0.06
Mix2	5.50 b ± 0.57	1.14 b ± 0.10	0.73 b ± 0.07
Mix3	6.32 b ± 1.03	1.30 ab ± 0.25	0.77 ab ± 0.08
F	8.46	2.16	2.36
Sig.	0.00	0.10	0.08

Table (10): Relative organs weight of rats in the studied groups.

The results given in Table (10) revealed that relative weight of liver in all treated groups were decreased significantly compared with positive group. According to kidney there were significantly difference between Mix (2 and 3) compared with positive group. While there was no significant difference between Mix (1) and negative group. Concerning relative weight of heart there were significant difference between treated groups compared with positive group except Mix (3).

Histopathological examination of heart:

Microscopically, the hearts of rats from groups (1) revealed the normal histological structure of cardiac myocytes (Figs. 7). Meanwhile, the heart of rats from group (2) revealed intramyocardial inflammatory cells infiltration (Fig. 8) and focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (Fig. 9). Moreover, heart from the group (2) showed intramyocardial inflammatory cells infiltration (Figs. 10). The heart of rats from the group (3) revealed no histopathological alterations (Fig. 11). Moreover, heart of rats from group (4) revealed no histopathological changes except slight intramyocardial oedema (Figs. 12). Additionally, heart of rats from group (4) revealed no histopathological changes (Figs. 13). Meanwhile, sections from group (5) showed intramyocardial inflammatory cells infiltration (Fig. 14) and focal necrosis of

cardiac myocytes associated with inflammatory cells infiltration (Fig. 15).

Fig. (7): Heart of rat from group 1 showing the normal histological structure of cardiac myocytes (H & E X 400).

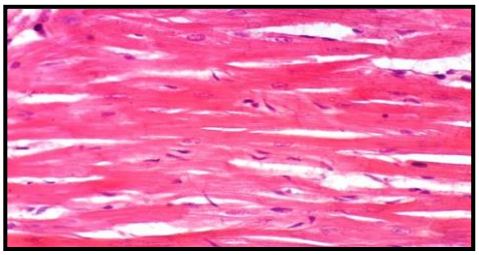


Fig. (8): Heart of rat from group 2 showing intermyocardial inflammatory cells infiltration (H & E X 400).

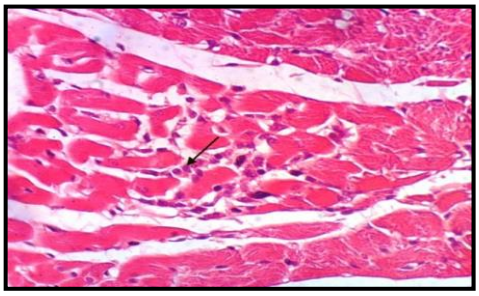


Fig. (9): Heart of rat from group 2 showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).

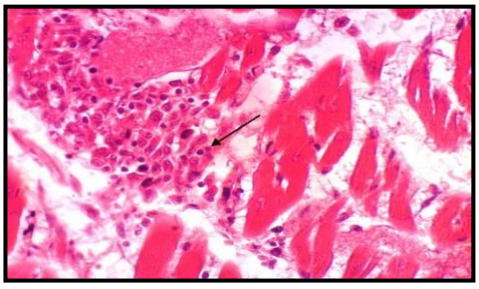


Fig. (10): Heart of rat from group 2 showing intermyocardial inflammatory cells infiltration (H & E X 400).

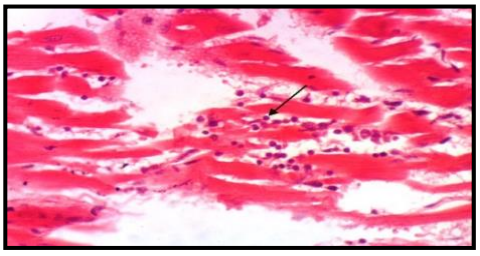


Fig. (11): Heart of rat from group 3 showing no histopathological alterations (H & E X 400).

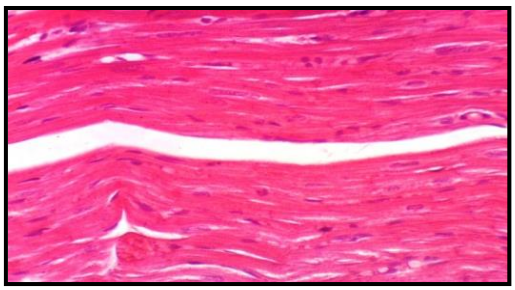


Fig. (12): Heart of rat from group 4 showing slight intermyocardial oedema (H & E X 400).

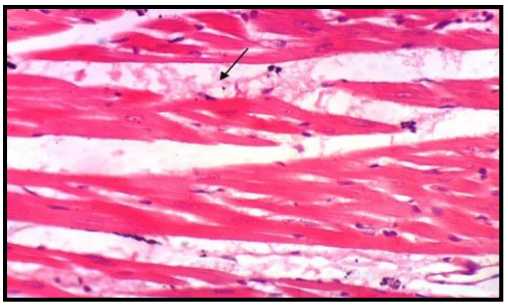


Fig. (13): Heart of rat from group 4 showing no histopathological alterations (H & E X 400).

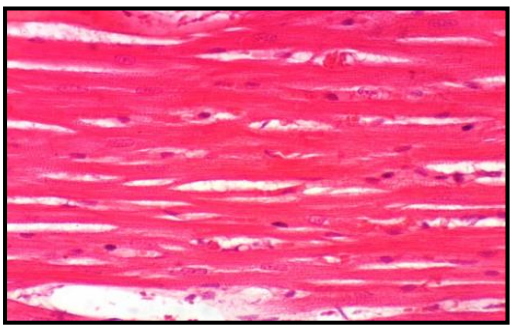


Fig. (14): Heart of rat from group 5 showing intermyocardial inflammatory cells infiltration (H & E X 400).

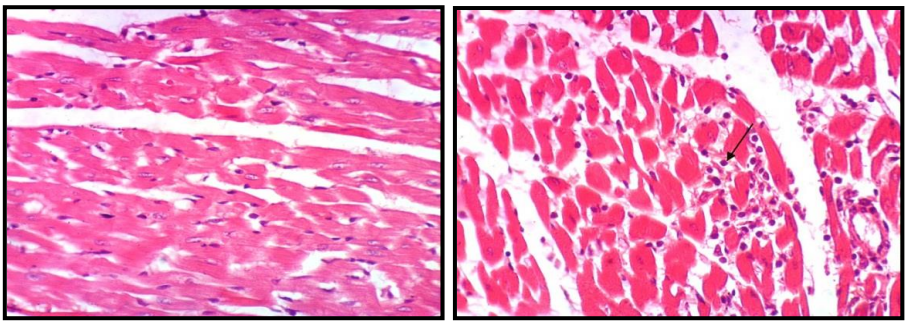
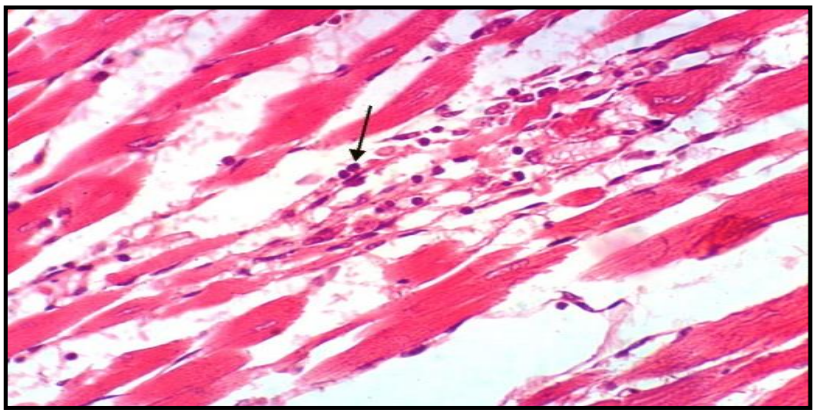


Fig. (15): Heart of rat from group 5 showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).



Histopathological examination of kidneys:

Microscopically, the kidneys of rats from the group (1) revealed the normal histological structure of renal tissue (Fig. 16). On the other hand, the Kidneys of rats from the group (2) showed vacuolar degeneration of epithelial lining renal tubules (Figs. 17 and 18). Meanwhile, sections from the group (3) showed no histopathological changes (Fig. 19). On the other hand, sections from the group (4) showed slight congestion of glomerular tufts (Fig. 20) and focal necrosis of renal tubules associated with inflammatory cells infiltration (Fig. 21). Meanwhile, congestion of renal blood vessels was the only change observed in kidneys from the group 4 (Figs. 22). Some kidneys of rats from the group (5) showed focal necrosis of renal tubules associated with inflammatory cells infiltration (Fig. 23), However, kidneys from group (5) slight congestion of glomerular tufts (Figs. 24 and 25).

(Fig. 16): Kidney of rat from group 1 showing normal histological structure of renal tissue

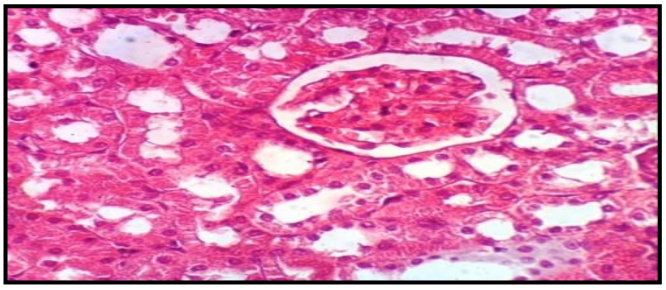


Fig. (17,18): Kidney of rat from group 2 showing vacuolar degeneration of epithelial lining renal tubules (H & E X 400).

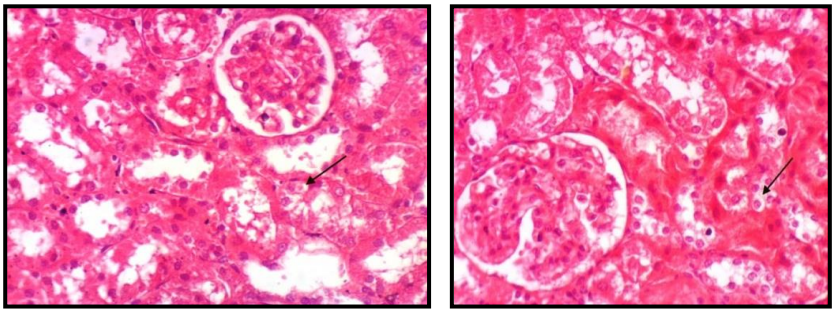


Fig. (19): Kidney of rat from group 3 showing no histopathological changes (H & E X 400).

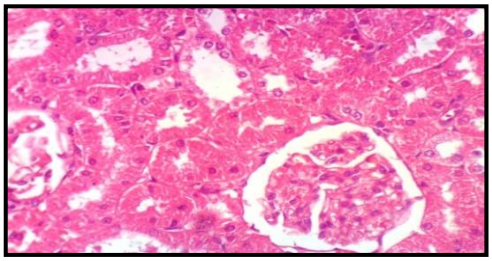


Fig. (20): Kidney of rat from group 4 showing slight congestion of glomerular tufts (H & E X 400).

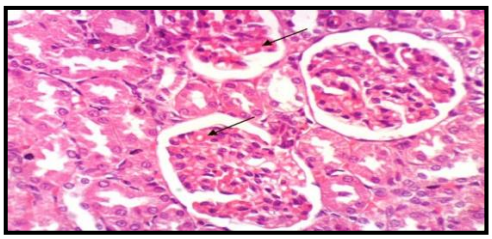


Fig. (21): Kidney of rat from group 4 showing focal necrosis of renal tubules associated with inflammatory cells infiltration (H & E X 400).

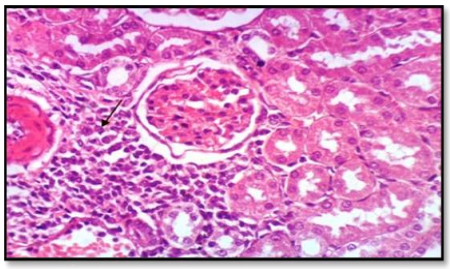


Fig. (22): Kidney of rat from group 4 showing congestion of renal blood vessel (H & E X 400).

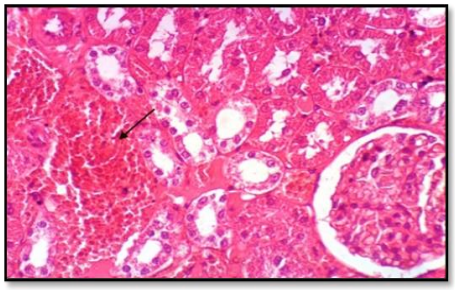


Fig. (23): Kidney of rat from group 5 showing focal necrosis of renal tubules associated with inflammatory cells infiltration (H & E X 400).

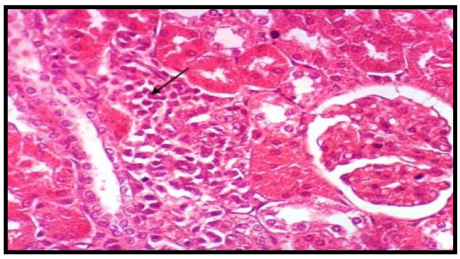
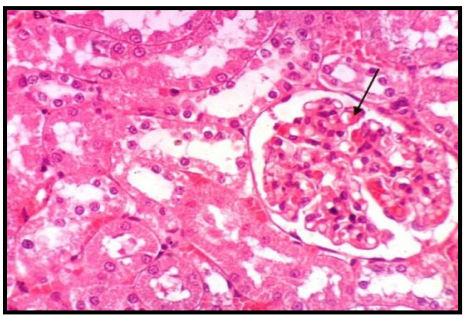
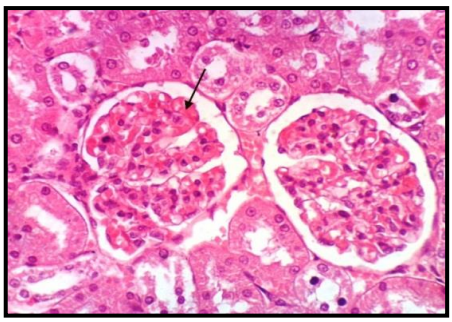


Fig. (24,25): Kidney of rat from group 5 showing slight congestion of glomerular tufts (H & E X 400).





Histopathological

examination of liver:

Microscopically, the liver of rats from the group (1) revealed the normal histological structure of the hepatic lobule (Fig. 26). On the other hand, liver of rats from the group (2) revealed steatosis of hepatocytes (Fig. 27), fibroplasia (Figs. 28) and inflammatory cells infiltration in the portal triad (Figs. 29). Moreover, liver from the group (2) also showed vacuolar degeneration of hepatocytes (Fig. 30) and sinusoidal leukocytosis (Fig. 31). However, the liver of rats from the group (3) revealed no histopathological alterations (Fig. 32), whereas other sections from this group showed sinusoidal leukocytosis and portal inflammatory cells infiltration (Fig. 33). Examined sections from the group (4) showed slight proliferation of Kupffer cells (Fig. 34) and small focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (Fig. 35). Meanwhile, liver from the group (4) revealed no histopathological alterations (Fig. 36). However, the liver from the group (5) showed sinusoidal leukocytosis (Fig. 37) and portal inflammatory cells infiltration (Fig. 38). No histopathological alterations were noticed in the liver from the group 5 (Figs. 39 and 40).

Fig. (26): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H& E X 400).

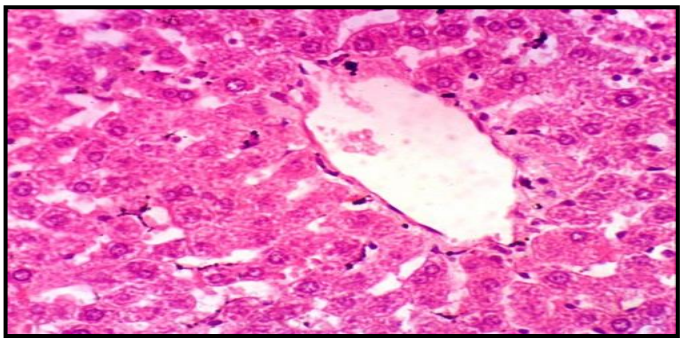


Fig. (27) : Liver of rat from group 2 showing steatosis of hepatocytes (H & E X 400).

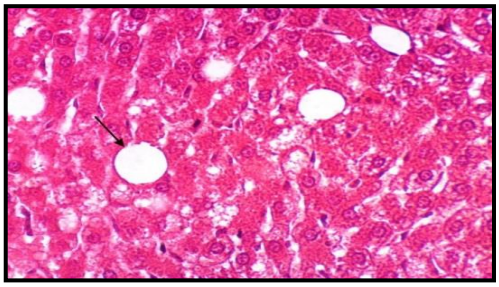


Fig. (28): Liver of rat from group 2 showing fibroplasia and inflammatory cells infiltration in the portal triad (H & E X 400).

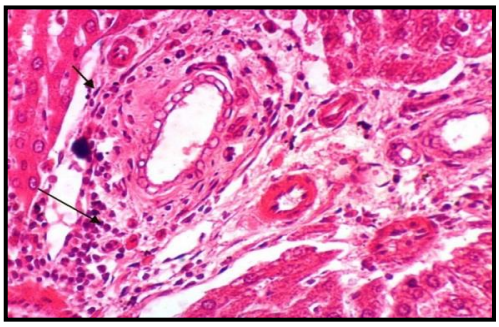


Fig. (29): Liver of rat from group 2 showing hyperplasia of biliary epithelium and inflammatory cells infiltration in the portal triad (H & E X 400).

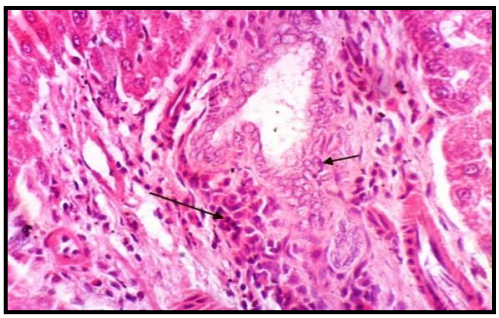


Fig. (30): Liver of rat from group 2 showing vacuolar degeneration of hepatocytes (H & E X 400).

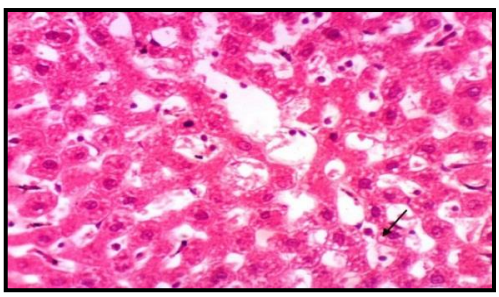


Fig. (31): Liver of rat from group 2 showing vacuolar degeneration of hepatocytes and sinusoidal leukocytosis (H & E X 400).

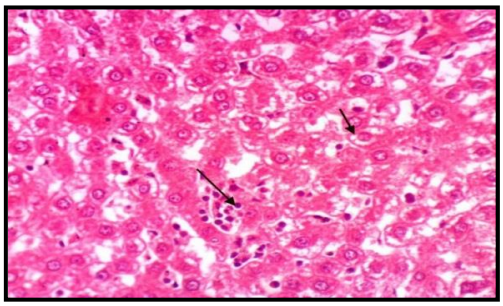


Fig. (32): Liver of rat from group 3 showing no histopathological alterations (H & E X 400).

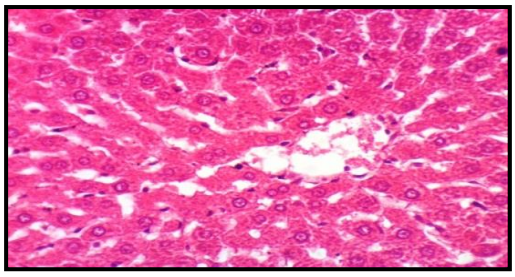


Fig. (33): Liver of rat from group 3 showing sinusoidal leukocytosis and portal inflammatory cells **infiltration (H & E X 400).**

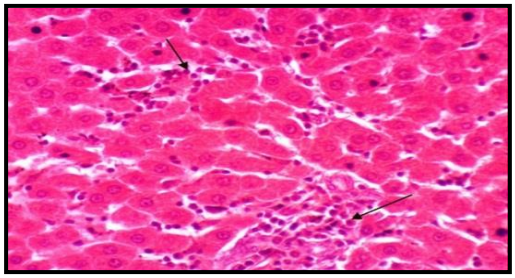


Fig. (34): Liver of rat from group 4 showing slight proliferation of Kupffer cells (H & E X 400).

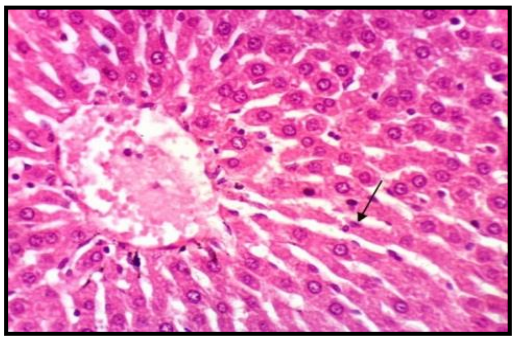


Fig. (35): Liver of rat from group 4 showing small focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (H & E X 400).

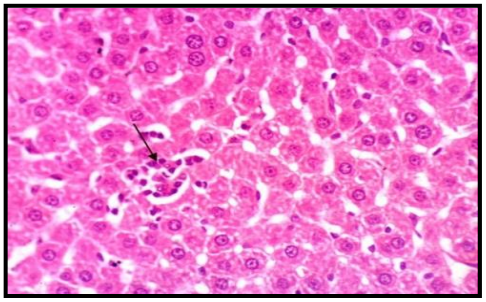


Fig. (36): Liver of rat from group 4 showing no histopathological alterations (H & E X 400).

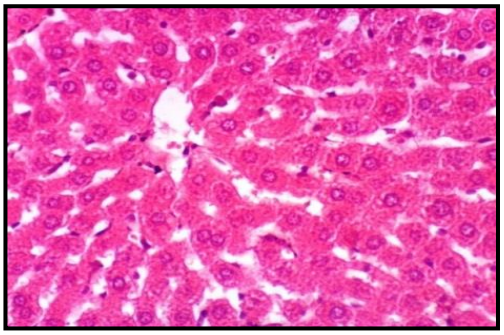


Fig. (37): Liver of rat from group 5 showing sinusoidal leukocytosis (H & E X 400).

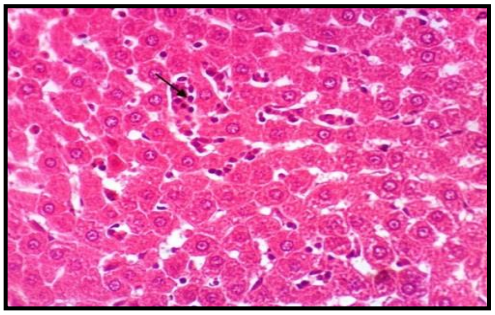
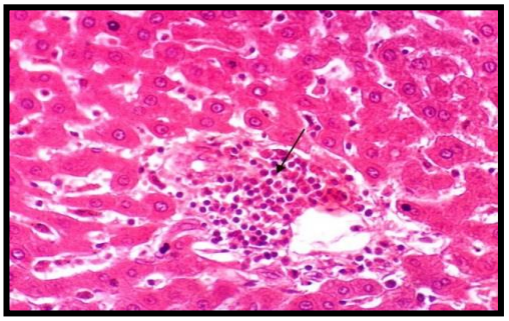
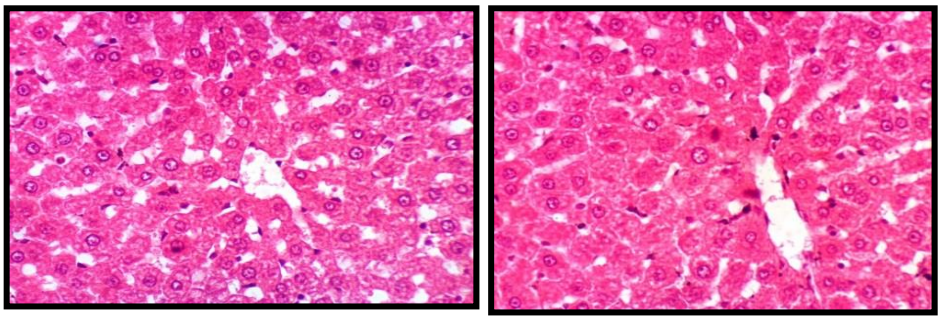


Fig. (38): Liver of rat from group 5 showing portal inflammatory cells infiltration (H & E X 400).



Figs. (39,40): Liver of rat from group 5 showing no histopathological alterations (H & E X 400).



Discussion

The current study aimed to evaluate the effect of mixing some spices (cinnamon, cardamom, turmeric, ginger, and clove) on biochemical parameters and body weight gain in obese rats. Various studies have been conducted to verify the effectiveness of Spices like cinnamon, cloves, ginger, and turmeric in treating metabolic diseases and their related disorders. These studies had provided a significant effect in improving these disorders.

The present study investigates whether mixing several medical spices together will provide a more significant effect in treating obesity, hyperlipidemia, and high blood glucose levels or not. And determine which mix of them is more effective.

Consumption of a high-fat diet causes leading obesity and obesity-related complications. It is known to be a risk factor for numerous metabolic complaints such as diabetes, atherosclerosis, hyperlipidemia, and cancer ***43.*** Natural products can show an obvious role in the prevention of obesity and associated metabolic diseases. Cinnamon has been used as spice for a long time also Research in animal models has also proven that cinnamon effectively prevents obesity caused by high fat diets ***44, 45*** The compounds in herbs and spices help enhance metabolism and deter against obesity, diabetes, and chronic inflammation. Many herbs and spices have positive effects on blood glucose, insulin

sensitivity, dyslipidemia, weight gain and the cardiovascular system ***46.***

Spices like cinnamon, cloves, turmeric, and bay leaves have insulin-potentiating In vitro and animal. data demonstrate that cinnamon increases insulin receptor sensitivity and/or directly stimulates insulin-producing cells. ***47, 48.***

there was a significant difference in Chemical composition between the tested samples**, mix 1** (cinnamon, ginger, and cardamom) was the highest content of crude fiber compared with mix 2 and mix 3. While it was the lowest content of protein, fat, ash, and moister compared with mix 2, and mix 3. This may be referring to cinnamon, ginger, and cardamom high content of nutrients. Beside its high content of soluble fiber. also, there was a significant difference in Mineral's content between the tested samples**, mix 1** was the highest contain of Calcium Ca (5.353mg/kg.) and Zink Zn (14.43 mg/kg.), compared with **mix 2** (2.633 mg/kg., and 2.69 mg/kg respectively) and **mix 3**(4.312 mg/kg, and 11.27 mg/kg respectively). while **mix 2** was the highest contain of potassium K (2.53 %) and Sodium Na (1.260.00 mg/kg.), compared with **mix3**(2.15%, and 701.30 mg/kg. respectively) and **mix 1** (1.84%, and 306.20 mg/kg. respectively) Whereas the contain of Iron Fe was the highest in mix3 (290.20 mg/kg) compared with mix 2 (287.30 mg/kg) and mix 1 (142.90 mg/kg) respectively.

also **mix 1** (cinnamon, ginger, and cardamom) was reported a highly contain monounsaturated fatty acids (omega 9 fatty acids ω9) and polyunsaturated fatty acids (omega 6 (ω6), and omega 3 (ω3), fatty acids) also, a highly content (omega 7 ω7 fatty acids), whereas has a low contain of saturated fatty acids compared with mix2 and mix 3.

Natural antioxidants like polyphenols and flavonoids neutralize oxygen free radicals (as hydroxyl radicals, superoxide radicals and other active oxygen species also including singlet

oxygen) and protect lipids, proteins, and DNA from oxidative damage, thus reducing the risk of obesity disease and cancer.

The results revealed that according to the mixture of (cinnamon, ginger, and cardamom). They contain many polyphenolic compounds that are considered to possess significant antioxidant activity. Antioxidant effects of cinnamon and cardamom were explored in rats fed a fat diet and it was found that antioxidant activities of enzymes were significantly increased, and glutathione content was manifestly restored. Besides, these spices were found somehow to check the increment in lipid conjugated dienes as well as hydroperoxides that are the chief products of lipid peroxidation. Antioxidant activity of cinnamon and cocoa extract, and the interaction of their mixtures using different in vitro tests. The outcomes exhibited a significant rise in the antioxidant activity of the cocoa extract through the addition of the cinnamon extract these imply that phytochemical components of mix (1) such as sterols, phenolic acids (catechein and ellagic acid) may promote the antioxidant defense system and scavenge free radicals. ***49.***

Concerning mixture (ginger, clove, and curcumin) It was indicated that curcumin as a main component of turmeric, prevents the formation of reactive oxygen species and reactive nitrogen species and scavenges them. In addition, it induces several enzymatic antioxidants, like glutathione transferase, haeme-oxygenase-1, and catalase. Ginger inhibited the oxidative stress and inflammation by enhancing antioxidant enzymes and decreasing inflammatory TNF-α level) ***50.***

Effect of the three mixes on Biochemical Parameters

animals treated with the three mixes show a significant decrease in total Cholesterol, Triglyceride, and LDL-c, and a significant increase in HDL-c. Especially the group fed on **mix 1** (5g cinnamon+ 5g ginger + 3 g cardamom /kg b.wt.). reported a great improvement in Cholesterol, Triglyceride, HDL-c, and LDL-c, compared with groups fed on **mix 2**. (5g Turmeric + 5g

ginger + 3 g clove /kg b.wt.) or **mix 3** (5g cinnamon+ 5g ginger + 3 g cardamom +5g Turmeric + 3 g clove /kg b.wt.). this result refers to **mix 1** high content of crude fiber (16.60%), compared with mix 2 and mix 3. in addition its high contain of polyphenols such as salyclic acid, catechein, ellagic acid, rosemarinic, rutin, ferulic acid, o.coumaric acid and pyrogallo. The possible hypolipidemic effect of mix 1 could be due to its high contents of polyphenols in cinnamon and of gingerols in ginger which inhibit the intestinal absorption of cholesterol with subsequent hypocholesterolemic activity.

(Couturier, et al., 2011) reported that experimental and clinical studies have shown that cinnamon could be attributed to its beneficial effects on hyperlipidemia. **51.** Al Jamal 2009 investigated the effects of cinnamon on type 1 diabetic individuals consumed (6g/d) of cinnamon for 4 weeks lower the mean of triglyceride (36%), LDL (30%) and total cholesterol (30%) **26.** also, Qin, et al 2004 showed that Cinnamon, is rich in polyphenolic components that improve the action of insulin in animal studies **52.** Ginger has been known to have antioxidant activity due to the presence of gingerol-related compounds. The results of Reza, et al., 2008 shows that ginger has a significant lipid lowering effect compared to placebo **53**. while Najmeh, et al., 2019 demonstrated that ginger intake reduced BW, WHR, HR, fasting glucose and HOMA-IR, and increased HDL-cholesterol, but did not affect insulin, BMI, triglycerides, total- and LDL-cholesterol levels **54.**

Azimi et al., 2014 reported that after 8 weeks of intervention, cinnamon, cardamom, ginger, and saffron consumption had significant effects on total cholesterol, LDL, and HDL levels ($p < 0.05$) compared with controls **55.** Maskooni, et al., 2019 showed that the green Cardamom supplement improved the grade of fatty liver, serum glucose indices, lipids, and irisin level among overweight or obese NAFLD patients **56.** Aghasi, et al., 2019 reported that intake of green cardamom led to a considerable reduction in serum TG levels **57.** Verma et al.2012

reported that intake of 3 g/d cardamom for 12 weeks in patients with ischemic heart disease decreased blood lipids (except HDL-c) **58.**

Effect of the three mixes on serum glucose level

in animals shows a significant decrease in serum glucose level in the groups treated with the three mixes. Several studies have reported that clove exerts a variety of pharmacological actions, including antioxidant, hypoglycemic and anti-inflammatory activities **59.** Setty, et al., 2005 suggest that a combination of turmeric, ginger, boswellia, and ashwaganda reduces inflammation **60.** (Saraswat et al., 2010), reported that Ginger is thermogenic, antioxidative, stimulating, anti-inflammatory, anti-hyperglycemic and inhibited glycation **61.** Yiran, et al., 2017 found that Fasting Blood Glucose analysis showed a significant lower in the Alcoholic extract Clove diet group compared with the HFD diet group **29**. Selvi, et al., 2015 found that Turmeric supplementation in metformin treated type 2 diabetic patients significantly decreased fasting glucose **62.** This resalt disagree with Wickenberg, et al., 2010 who reported that ingestion of one dose of 6 g Turmeric increased postprandial serum insulin levels but did not affect plasma glucose levels or glycemic index, in healthy subjects **63**. Perez, et al 2013 found that Turmeric alone (2.4 g/day), had no effect on the blood glucose without significant effect on blood glucose with 2 g of Turmeric powder taken for 8 weeks **64**. Azimi et al., 2014 reported that, the herbal products have no significant effects on measures of glycemic control, anthropometry, inflammation, and oxidative stress. In within-group comparisons only, cinnamon intake significantly decreased fasting blood sugar **55**. Maskooni, et al., 2019 showed that the green Cardamom supplement improved the serum glucose indices among overweight or obese NAFLD patients **56.**

Effect of the three mixes on body weight gain (BWG)

all groups treated with the three mixes show a significant decrease in (BWG). Especially the group fed on mix 3 reported a

great decrease (-36.63) compared with groups fed on mix 1 or mix 2. Vafa, et al., 2012 found positive effects of 3 g cinnamon per day on the body weight of diabetic patients **65.** Yiran, et al., 2017 found that mice fed a high-fat diet supplemented with Alcoholic extract Clove for 14-weeks showed a reduction in body weight, epididymal and perirenal fat pad weight and liver weight as compared to mice fed the HFD diet alone **29**. Atashak, et al., 2011 reported that Ginger supplementation and progressive resistance training were also shown to decrease waist circumference significantly in obese men **66.**

Effect of the three mixes on Liver & kidney function

Concerning GPT results of the present study indicated that there were no significant differences between treated groups compared positive group. While there were significant differences between Mix (2&3) compared with positive group for GOT. These may be related to the mixture of (cinnamon, ginger &turmeric) which can be used as a safe and inexpensive agent to improve body weight, lipid profiles, insulin resistance, and blood pressure and serum liver enzymes. This result was disagreement with (Sahebkar, 2011) that oxidative stress and immune system disorder play important roles in contributing to liver dysfunction cinnamon can improve oxidative stress and prevent NAFLD by decrease production of reactive oxygen species, the hepatic protein expression of oxidative stress, pro-inflammatory cytokines, and chemokines. The reduction the effect of cinnamon on GPT and GOT levels was observed **67.**

Regarding to urea it was obvious that Mix (1) supplementation reduced serum urea in obese rat compared with positive control (44.77 and 52.27). While mix (1and 2) supplementation increased serum creatinine compared to negative group (1.23, 1.19 and 0.99) respectively. These results agreed with (Piccoli et al., 2018). who revealed that neither supplemented with herbs extract nor fresh affected serum creatinine concentration measured 12 weeks after fat diet induction compared to control rats, demonstrated significantly

increased serum urea concentration measured 12 weeks after induction ($p \leq 0.001$) **68**.

Relative organs weight of rats in the studied groups

There was a significantly difference between Mix (2 and 3) compared with the positive group in the relative weight of kidney, while there was no significant difference between Mix (1) and negative group. Concerning relative weight of heart there was a significant difference between treated groups compared with positive group. Data obtained from the organ weigh variations showed that the average weight of heart was decreased remarkably in all groups when compared with control except Z. officinale Roscoe high-fat diet group (weight was like control). The average weight of the liver followed the same manner. The average weight of the kidney was found to significantly decrease in A. indica A.Juss normal and high-fat diet group ($p < 0.01$) but increased in both group of Z. officinale Roscoe and T. foenum-graecum L. high-fat diet group **69.**

Histopathological findings of rats in the studied groups

Our results are confirmed by the histopathological findings which revealed remarkable steatosis hepatocytes, fibroplasia, and inflammatory cells infiltration in the positive group. While according to mix (1) supplementation it was observed that no pathology findings and other sections sinusolidal alteration. Concerning mix (2) Kupffer cells activation, small focal hepatocellar necrosis was also recorded in all examined sections of liver in group (4). But supplemented mix (3) reported sinusoidal leukocylosis were observed. These results were consistent with that reported by Hinson et al. (2010) **70**; Hamza and Al-Harbi, (2015) **71**.

References

1. Tobore, O.T,(2020). Towards a comprehensive theory of obesity and a healthy diet: The causal role of oxidative stress in food addiction and obesity. Behavioral Brain Research 384 ,112560.
2. Abdelkarim, Osama, Achraf Ammar, Asmaa M A Soliman, and Anita Hökelmann. 2017. "Egyptian Pediatric Association Gazette Prevalence of Overweight and Obesity Associated with the Levels of Physical Fitness among Primary School Age Children in Assiut City." Egyptian Pediatric Association Gazette 65(2): 43–48.
3. Ajayi, Elizabeth Onazahi, Hassan Abdullahi Elechi, and Mohammad Arab Alhaji. 2015. "Prevalence of Overweight / Obesity among Primary School Pupils in Urban Centre, Nigeria." Saudi Journal of Obesity 3: 59–65.
4. Webber.L, D. Divajeva, T. Marsh, K. McPherson, M. Brown, G. Galea, J. Breda. (2014) "The future burden of obesity-related diseases in the 53 WHO European-Region countries and the impact of effective interventions: a modelling study", BMJ 4787.
5. Kazemipoor, M., Cordell, G. A., Sarker, M. M. R., Radzi, C. W. J. B. W. M., Hajifaraji, M., &Kiat, P. E. (2015). Alternative treatments for weight loss: Safety/risks and effectiveness of anti-obesity medicinal plants. International Journal of Food Properties,18(9), 1942–1963.
6. Fu, C. H., Jiang, Y., Guo, J., & Su, Z. Q. (2016). Natural products with anti-obesity effects and different mechanisms of action. Journal of Agricultural and Food Chemistry, 64,9571–9585.
7. Embuscado, M. E. (2015). Spices and herbs: Natural sources of antioxidants–a mini review.Journal of Functional Foods, 18, 811–819.
8. Codex Alimentarius Comission (2014). FAO/WHO food standards programme. Code of hygienic practices for spices and dried aromatic plants.
9. Duke, J. A. (2002). CRC handbook of medicinal spices. Boca Raton, Florida, US: CRC Press.
10. Henning, S.M., Zhang, Y., Seeram, N.P., Lee, R.-P., Wang, P., Bowerman, S., Heber, D., (2011). "Antioxidant capacity and phytochemical content of herbs and spices in dry, fresh and blended herb paste form. Int. J. Food Sci. Nutr. 62 (3), 219–225.
11. Vallverdú-Queralt, A., Regueiro, J., Martínez-Huélamo, M., Alvarenga, J.F.R., Leal, L.N., Lamuela-Raventos, R.M., (2014). "A comprehensive study on the phenolic profile of widely used culinary herbs and spices: rosemary, thyme, oregano, cinnamon, cumin and bay. Food Chem. 154, 299–307.

12. Gonzalez. M, (2011). Dietary phytochemicals and their potential effects on obesity. Pharmacological Research 64. 438– 455.
13. MacLean PS, Wing RR, Davidson T, Epstein L, Goodpaster B, Hall KD, et al. NIH working group report (2015). innovative research to improve maintenance of weight loss. Obesity 23(1): 15.
14. Gupta, S. C., Sung, B., Kim, J. H., Prasad, S., Li, S., & Aggarwal, B. B. (2013). Multitargeting by turmeric, the golden spice: From kitchen to clinic. Molecular Nutrition & Food Research, 57, 1510–1528
15. Fan-Cheng Meng, Yan-Qing Zhou, Dai Ren, Ruibing Wang, et al., (2018). "Turmeric: A Review of Its Chemical Composition, Quality Control, Bioactivity, and Pharmaceutical Application." Natural and Artificial Flavoring Agents and Food Dyes.299-310.
16. Kumar K, Issac A, Ninan E, Kuttan R, Maliakel B. (2014). Enhanced anti-diabetic activity of polyphenol rich de-coumarinated extracts of Cinnamomum cassia. J Funct Foods.10:54.
17. Sedighi M, Nazari A, Faghihi M, Rafieian-Kopaei M, Karimi A, Moghimian M, et al., (2018). Protective effects of cinnamon bark extract against ischemia-reperfusion injury and arrhythmias in rat. Phytother Res 1:9.
18. Askari F, Rashidkhani B, Hekmatdoost A., (2014). Cinnamon may have therapeutic benefits on lipid profile, liver enzymes, insulin resistance, and high-sensitivity C-reactive protein in nonalcoholic fatty liver disease patients. Nutr Res;34(2): 14:38.
19. Wang J, Ke W, Bao R, Hu X, Chen F., (2017). Beneficial effects of ginger Zingiber officinale roscoe on obesity and metabolic syndrome: a review. Ann N Y Acad Sci; 1398:83–98.
20. Ali, B.H., Blunden, G., Tanira, M.O., Nemmar, A., (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 46, 409–420.
21. Khandouzi, N., Shidfar, F., Rajab, A., Rahideh, T., Hosseini, P., Taheri, M.M., (2015). The effects of ginger on fasting blood sugar, hemoglobin A1c, apolipoprotein B, apolipoprotein AI and malondialdehyde in type 2 diabetic patients. Iran. J. Pharm. Res. (IJPR) IJPR 14 (1), 131.
22. Abdali, H., Ajji, A., (2015). Development of antibacterial structures and films using clove bud powder. Ind. Crop. Prod. 72, 214–219
23. Heredia-Guerrero, J.A., Ceseracciu, L., Guzman-Puyol, S., Paul, U.C., Bayer, I.S., (2018). Antimicrobial, antioxidant, and waterproof RTV silicone-ethyl cellulose composites containing clove essential oil. Carbo. hydr. Polym.192, 150–158.

24. Abbasipour H, Mahmoud M, Rastegar F, Hosseinpour, MH., (2011). Fumigant toxicity and oviposition deterrence of the essential oil from cardamom, Elettaria cardamomum, against three stored–product insects. J Insect Sci 11(165):1–10.
25. Patel, MA., (2011) Effect of seed extracts of Elettaria cardamomum on calcium oxalate crystallization. Int J Pharm Res Technol 1(1):21–25.
26. Al Jamal, Abdul Rahim (2009): "Effects of cinnamon on blood glucose and lipids levels in diabetic patients (Type1) African Journal of Biochemistry Research Vol.3 (5), pp. 181-184,
27. Roussel A.-M., Hininger I., Benaraba R., Ziegenfuss T. N., and Anderson R. A.: (2009) Antioxidant Effects of a Cinnamon Extract in People with Impaired Fasting Glucose That Are Overweight or Obese. Journal of the American College of Nutrition, Vol. 28, No. 1, 16–21
28. Kabaran S., and Atakan A., (2018): Effects Of Dietary Intervention with or without Turmeric on Blood Lipids and Weight Loss in Dyslipidemic overweight /Obese Women. Clinical Nutrition 37 S46eS314
29. Yiran Ding, Zhennan Gu, Yihe Wang, Shunhe Wang, Haiqin Chen, Hao Zhang, Wei Chen, and Yong Q. Chen., (2017): Clove extract functions as a natural fatty acid synthesis inhibitor and prevents obesity in a mouse model Food Funct, 8, 2847–2856
30. AOAC (Association of Official Analytical Chemists) (2005). Official methods of analysis (18th ed.). Arlington, VA: AOAC. International 30–31 Chapter 4.
31. Cossignani, L, Simonetti, MS and Damiani, P. 2005. Biocatalyzed acidolysis of olive triacylglycerols with 9c, 11t and 10t, 12c isomers of conjugated linoleic acid. Eur Food Res. Technol. 220, 267-271.
32. Eid, J., A. Fehr, et al. (2009). “Real-Time DNA Sequencing from Single Polymerase Molecules.” Science 323
33. (5910): 133-138
34. Tuzcu Z., Orhan C., Sahin N., Juturu V., and Sahin K., 2017: Cinnamon Polyphenol Extract Inhibits Hyperlipidemia and Inflammation by Modulation of Transcription Factors in High-Fat Diet-Fed Rats. Oxidative Medicine and Cellular Longevity, Volume 2017, Article ID 1583098, 10 pages. https://doi.org/10.1155/2017/1583098
35. Koditscheck, L.K. and Umbreit, W.W. (1969): Triglycerides. Gpo-PAP Method, J. Bacteriol, vol. 98, pp. 1063-68.

36. Richmond, W. (1973): Preparation and properties of a cholesterol oxidase from nocardial SP. And its application to the enzyme assay of total cholesterol. Sprum Clin. Chem., vol. 19, pp. 1350-56.

37. Levy, R. T. (1981): Cholesterol; lipoprotein, present status, and future prospects. Clin. Chem., vol. 27, pp. 653.
38. Burstein, M. (1970): HDL- cholesterol determination after separation of high-density lipoprotein lipid. J. Lip. Res., vol. 11, pp. 583.
39. Wieland, K.L. and Seidel, D. (1983): LDL-cholesterol: fully enzymatic colorimetric test, J. Lipid Res., vol. 24, pp.904.

40. Reitman S and Frankel S, (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
41. Fossati P, Prencipe L, Berti G (1980). Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin.Chem., 26: 227-231
42. Fawcett JK, Scott JE (1960). A rapid and precise method for the determination of urea. J. Clin. Pathol., 13: 156-159
43. Szasz G, Borner U, Busch EW , Bablok W (1979). [Enzymatic assay of creatinine in serum: comparison with Jaffe methods (author's transl)]. J. Clin. Chem. Clin. Biochem., 17: 683-687.
44. Bancroft JD, Stevens A (2008). Theory and practice of Histology Technique 6th Edition. Edinburgh: Churchill Livingstone. Philadelphia, PA, pp, 135-217.
45. Son Y., Nam J.-S., Jang M.-K., Jung I.-A., Cho S.-I., and Jung M.-H., 2013. “Anti-obesity activity of Vigna nakashimae extract in high-fat diet-induced obesity,” Bioscience, Biotechnology, and Biochemistry, vol. 77, no. 2, pp. 332–338,
46. Beejmohun V., Peytavy-Izard M., Mignon C. et al., 2014 “Acute effect of Ceylon cinnamon extract on postprandial glycemia: alpha-amylase inhibition, starch tolerance test in rats, and randomized crossover clinical trial in healthy volunteers,” BMC Complementary and Alternative Medicine, vol. 14, article 351.
47. Boqu´e N., Campi´on J., de la Iglesia R. et al., (2013) “Screening of polyphenolic plant extracts for anti-obesity properties in Wistar rats,” Journal of the Science of Food and Agriculture, vol. 93, no. 5, pp. 1226–1232,
48. Jungbauer, A., & Medjakovic, S. (2012). Anti-inflammatory properties of culinary herbs and spices that ameliorate the effects of metabolic syndrome. Maturitas, 71(3), 227e239.

49. Khan A, Bryden NA, Polansky MM, Anderson RA (1990). Insulin potentiating factor and chromium content of selected foods and spices. Bio. Trace. Element Res. 24: 183-188.
50. Verspohl EJ, Bauer K, Neddermann E. (2005) "Antidiabetic effect of Cinnamomum cassia and Cinnamomum zeylanicum in vivo and in vitro. Phytother Res.; 19:203-206.
51. Muhammad, D.R.A., Praseptiangga, D., Van de Walle, D., Dewettinck, K., (2017). Interaction between natural antioxidants derived from cinnamon and cocoa in binary and complex mixtures. Food Chem. 231, 356–364.
52. Si, W., Chen, Y. P., Zhang, J., Chen, Z.-Y., & Chung, H. Y. (2018). Antioxidant activities of ginger extract and its constituents toward lipids. Food Chemistry, 239, 1117–1125.
53. Couturier K., Qin B., Batandier C. et al., (2011): "Cinnamon increases liver glycogen in an animal model of insulin resistance," Metabolism: Clinical and Experimental, vol. 60, no. 11, pp. 1590–1597
54. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y (2004): Cinnamon extract prevents the insulin resistance induced by a high fructose diet. Horm Metab Res 36:119–125,
55. Reza A. N., Fatemeh R., Mehrdad S., Mehdi P., Farzad J., Ali A. M. (2008): Investigation of the effect of ginger on the lipid levels. A double blind controlled clinical trial. Clinical Trial Saudi Med J. (9):1280-4
56. Najmeh M., Reza T., Kamran B. L., Abbas R., Maryam A., Fariba K, Maryam R., Fariba K. & Zatollah A., (2019): The effects of ginger intake on weight loss and metabolic profiles among overweight and obese subjects: A systematic review and meta-analysis of randomized controlled trials, Critical Reviews in Food Science and Nutrition. Vol. 59, 11
57. Azimi P, Ghiasvand R, Feizi A, Hariri M and Abbasi B, (2014): Effects of cinnamon, cardamom, saffron, and ginger consumption on markers of glycemic control, lipid profile, oxidative stress, and inflammation in type 2 diabetes patients. Rev Diabet Stud 11:258–266
58. Maskooni M. D., Keshavarz S. A., Qorbani M., Mansouri S., Alavian S. M., Fariman M. B., Tehrani S. A., Sotoudeh G., (2019): Green cardamom supplementation improves serum irisin, glucose indices, and lipid profiles in overweight or obese non-alcoholic fatty liver disease patients: a double-blind randomized placebo-controlled clinical trial. BMC Complement Altern Med Mar 12;19(1):59

59. Aghasi M., Koohdani F., Qorbani M., Esfahani E. N., Zahedi Sh. G.-, Khoshamal H., Keshavarz A. and Sotoudeh G., 2019: Beneficial effects of green cardamom on serum SIRT1, glycemic indices and triglyceride levels in patients with type 2 diabetes mellitus: a randomized double-blind placebo controlled clinical trial. Wiley Online Library: 18 March 2019
60. Verma SK, Jain V and Singh DP, (2012). Effect of greater cardamom (Amomum subulatum Roxb.) on blood lipids, fibrinolysis and total antioxidant status in patients with ischemic heart disease. Asian Pac J Trop Dis2:S739–S743
61. Setty AR, Sigal LH. (2005) Herbal medications commonly used in the practice of rheumatology: mechanisms of action, efficacy, and side effects. Semin Arthritis Rheum.;34: 773-784.
62. Saraswat, M., Suryanarayana, P., Reddy, P. Y., Patil, M. A., Balakrishna, N., & Reddy, G. B. (2010). Anti-glycating potential of Zingiber officinalis and delay of diabetic cataract in rats. Molecular Vision, 16, 1525e1537.
63. Selvi N. M. K., Sridhar M. G., Swaminathan R. P. & Sripradha R. (2015) Efficacy of Turmeric as Adjuvant Therapy in Type 2 Diabetic Patients. Indian Journal of Clinical Biochemistry volume 30, pages 180–186
64. Wickenberg J, Ingemansson SL, Hlebowicz J. (2010) "Effects of Curcumalonga (turmeric) on postprandial plasma glucose and insulin in healthy subjects. Nutr J;9(1):43.
65. Perez-Torres I, Ruiz-Ramirez A, Banos G, El-Hafidi M. 2013 Hibiscussabdariffa Linnaeus (Malvaceae), curcumin and resveratrol as alternative medicinal agents against metabolic syndrome. Cardiovasc Hematol Agents Med Chem;11(1):25—37.
66. Vafa M, Mohammadi F, Shidfar F, Sormaghi MS, Heidari I, Golestan B, Amiri F. (2012) "Effects of cinnamon consumption on glycemic status, lipid profile and body composition in type 2 diabetic patients". Int J Prev Med.;3(8):531–536
67. Atashak S, Peeri M, Azarbayjani MA, Stannard SR, Haghighi MM. (2011) "Obesity-related cardiovascular risk factors after long-term resistance training and ginger supplementation. J Sports Sci Med.;10(4):685–691
68. Sahebkar A (2011). Potential efficacy of ginger as a natural supplement for nonalcoholic fatty liver disease. World J Gastroenterol.;17(2):271.
69. Piccoli, G., Alrukhaimi, M., Liu, Z., Zakharova, E., Levin, A., Tao Li, P., Garcia-Garcia, G., Benghanem-Gharbi, M., Kalantar-Zadeh, K., Kernahan, C., Kumaraswami, L., Piccoli, G., Saadi, G., Fox, L.,

Andreoli, S., (2018). Women and kidney disease: Reflections on World Kidney Day. Dial. Transplant Nephrol.

70. Jamous,R.M. S.Y. Abu-Zaitoun, R.J. Akkawi, M.S. Ali-Shtayeh, (2018). Antiobesity and antioxidant potentials of selected palestinian medicinal plants, Evid. base Compl. Alternative Med.
71. Hinson JA, Roberts DW, James LP (2010). Mechanisms of Acetaminophen-Induced Liver Necrosis. Hand. Exp.Pharmacol. 196:369–405.
72. Hamza R.Z., Al-Harbi M.S. (2015). Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and Nigella sativa extract supplements. Asian Pac. J. Trop. Biomed. 5(7):521–531.