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Effect of Using Natural Bioactive Mixture Composed of Lemon, Onion and Garlic Juices on Weight and Lipid Profiles for Overweight Rats

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Abstract: The World Health Organization encourages using medicinal herbs and plants to substitute the use of chemicals to go back to nature. Both garlic and onion have been shown to have applications as antimicrobial, antithrombotic, antitumor, hypolipidaemic, antiarthritic and hypoglycemic agents. Lemon juice and lemon oil have been evaluated for antimicrobial action. This work aimed to investigate the biological effect of natural juice mixture composed of lemon, onion and garlic (LOG) on overweight rats. Forty male rats (150±10 g) were divided into two groups (20 rats each), the first[1st] group fed on normal diet all the period of the study, the second group fed on high fat diet for eight weeks until they become overweight, then every group divided into two subgroups (10 rats each), to become four groups as follows:G_1: control normal diet, G_2: normal diet with 1 ml natural mixture, G_3: control high fat diet and G_4: high fat diet with 1 ml natural mixture. The study was continued for another two months. The result showed that; natural mixture solution occurred a significantly ($P<0.05$) decreasing in their body weight for overweight rats. Also it caused a significantly ($P<0.05$) decreasing in WBC level, creatinine and urea in both normal and high fat diet groups, noticed reducing in level of triglyceride, cholesterol and VLDL, albumen, T. Bilirubin and glucose. On the other hand, natural mixture significantly (($P<0.05$) increased total protein and globulin. From the results obtained and under condition that available during carrying this work it can be mentioned that adding mixture of LOG to overweight rats has the benefit effect in reducing body weight gain and can controlled the lipid contents in the body.

Key words: Lemon · Onion · Garlic · Overweight rats · Lipid profiles

INTRODUCTION

There is an increasing attention to herbs, spices and medicinal plants as possible growth promoter's and additives references. These components have different active substances [1]. Also, it has antioxidation ability [2], antimicrobial activity [3] and enhancing digestion by stimulating endogenous enzymes [4]. The World Health Organization (WHO) encourages using medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to nature [5].

Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes [6].

Garlic has many bioactive components, including several sulfur-containing compounds such as alliin, diallyl sulfides and allicin may partly account for some effects of garlic [7]. These components are known to possess antibacterial [8], antifungal, antiparasitic, antiviral [9], antioxidant [10] and anticancer activities [11]. Allicin is a powerful plant chemical which decomposes to several volatile organosulfur compounds with bioactivities, allicin is enzymatically released from precursor form when the garlic crushed [12].

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Onion bulbs possess numerous organic sulphur compounds including Trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, spropylcycteine sulfoxides and cycloallicin, flavinoids, phenolic acids, sterols including cholesterol, stigma sterol, b-sitosterol, saponins, sugars and a trace of volatile oil compounds mainly of sulphur compounds. Also, most of the plant parts contain compounds with proven antibacterial, antiviral, antiparasitic and antifungal properties and have antihypertensive, hypoglycemic, antithrombotic, anti-hyperlipidemic, anti-inflammatory and antioxidant activity [13].

Both garlic and onion have been shown to have applications as antimicrobial, antithrombotic, antitumor, hypolipidaemic, antiarthritic and hypoglycemic agents. In recent years, extensive research has focused on the beneficial and medicinal properties of garlic and onions. There is an increasing interest on the use of these agents in the treatment and prevention of cardiovascular disease and cancer [14].

Lemon is a good source of potassium (145mg/100g); calcium (61mg); vitamin C (40 to 50mg/100g); vitamins A, B_1, B_2 and B_3; and 27 Kcal/100g [15]. Lemon peel contains phenolic compounds, such as flavonoids (flavanones, flavonols, flavones), phenolic acids (ferulic, p-coumaric and sinapic acids), as well as vitamin C (ascorbic acid) as noted by [16], which have been linked to anti-microbial [17], anti-cancer [18] and anti-oxidant properties [19].

Phenolic compounds are the most abundant secondary metabolites synthesized by plants through the shikimate pathway [20] as a response to external stresses, such as ultraviolet radiation, wounding, aggression by pathogens, parasites and predators; in addition, they contribute to the color of plants [21].

Also, lemon includes volatile oil (2.5% of the peel), limonene, alpha-terpinene, alpha-pinene, citral, coumarins, mucilage, pectins and bioflavonoids (mostly from pith and peel as noted by others [22-24]. Lemon juice and lemon oil have been evaluated for antimicrobial action. The oil shows some bacteriostatic and antiviral action thought to be due to citral and linalool content [22, 23]. In addition for that, lemon also contained some active antioxidant compounds such as flavonoids, iso-flavones, flavones, anthocyanins, coumarins, lignans, catechins and iso-catechins [25]. So, their use has become highly regulated due to the possible development of drug resistance in human pathogenic bacteria [26].

Garlic, onion and lemon juice is highly inhibitory to molds and fungi [27]. Also, it was noted that adding natural additive to feed well increases the antioxidant content and may have potentials as a natural antioxidant and thus inhibit unwanted oxidation processes [28].

So, this investigation aimed to study the biological effect of natural mixture composed of lemon, onion and garlic juice (LOG) on weight, blood constituents and lipid profiles in overweight rats

MATERIALS AND METHODS

This study was carried out in the animal house of the Post Graduate and Research institute.

Preparation the Natural Mixture for Feed Supplementary: Natural mixture composed of lemon, onion and garlic (LOG) juice at portions (1.00, 1.00 and 0.125 / L clean water), respectively. This juices were supplied by the Regional Centre for Food and Feed, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt (Patent No.: 27392, Cairo, Egypt 2016). Prior to the study, the FVJ was tested and several components are found like (2, 5-Di-tertbutylhydroquinone (DBHQ), Allicin, Allyl mercaptan, Flavonoids, Quercetin, hydroxybenzoic acid, D-Limonene, Aspartic acid, S-2-aminoethyl cysteine, DL-3-aminobutyric acid, Cis-4, 7, 10, 13, 16, 19-Docosahexaenoic acid. Also, it contained amino acid methionine, lysine, phenylalanine, serine and as well as minerals (copper, calcium, potassium, magnesium, zinc, iron, sulphur, selenium and chromium). It is composed of the juice of lemon, onion and garlic (LOG) at portions 1.00, 1.00 and 0.125/l clean water, respectively.

Experimental Animals: Forty male rats weighted (150±10 g) were obtained from the animal house of Post Graduate and research institute Animals received human care and had adequate stable diet and water *ad lipitum*. Animals were acclimatized to the laboratory conditions for two weeks before being experimented. All laboratory biological specimens and hazardous waste were disposed safely. After two weeks, rats divided into two groups (20 rats each), the first group fed on normal diet all the period of the study, the second group fed on high fat diet for eight weeks until they become overweight. High fat diet were prepared as fat represented 5% of the total diet by using 80% tallow and 20 % corn oil.

Experimental Design and Diet: After eight weeks every group divided into two subgroups (10 rats each) to become four groups as follows: G_1: control normal diet, G_2:

normal diet with 1 ml natural mixture, G_3: control high fat diet and G_4: high fat diet with 1 ml natural mixture. The study was continued for another two months. Rats were fed on conventional food all over the experimental period (sucrose 50%, casein 20%, corn starch 15%, corn oil 5%, cellulose 5%, mineral mix 3.5%, itamin mix 1%, DL-Methionine 0.3% and Choline bitartrate 0.2%) as described by National Academy of Sciences [29].

Body Weight and Organs Weight: Body weight of rats was recorded at the beginning, after 8 weeks and at the end of the experimental period. Animals were sacrificed by decapitation, then liver, kidney, brain, heart spleen, kidney, lungs and pancreas were immediately removed and weighed. Relative organ weights were calculated as g/100 g body weight.

Blood Sample: Blood samples were collected from the sacrificed animals in two separate tubes, one of them containing heparin. Plasma samples were obtained by centrifugation at 4000 rpm for 20 minutes, then samples were stored at –20°C until used for further analyses.

Biochemical Parameters and Enzyme Activities: Plasma samples were analyzed for glucose according to Kunst *et al*. [30]. (Meanwhile, uric acid and creatinine concentrations were measured according to the method of Lamb *et al*. [31]. Total protein concentration and albumin were determined utilized commercial test kits supplied by Bio diagnostic Company for Laboratory Services according to Doumas *et al*. [32]. Meanwhile, globulin and albumin/globulin ratio (A/G) were calculated. Total bilirubin was measured using the method of Wahlefeld and Bergmeyer [33]. Cholesterol and triglycerides (TG) were determined according to the methods of Tietz [34. High-density lipoprotein-cholesterol (HDL-c) was determined according to the methods of Sugiuchi *et al*. [35]. Low-density lipoprotein-cholesterol (LDL-c) was determined by the method of Pisani *et al*. [36] Very low-density lipoprotein-cholesterol (VLDL-c) was calculated automatically by Roche /Hitachi Cobas C systems. The activities of plasma aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Bergmeyer and Herder [37]. Alkaline Phosphatase (ALP) activity was determined according to Hillmann [38]. All the aforementioned parameters were measured using commercial kits, [Bio systems S.A. (Spain), Diamond (Germany) and Randox (United Kingdom)].

Statistical Analysis: Data collected of weight, …… and blood parameters were subjected to statistical analysis as one-way analysis of variance according to SPSS [39]. Duncan's Multiple Range Test Duncan [40] was used to separate means when the dietary treatment effect was significant according to the following model:

$$Y = \mu + T + e$$

where:
Y = Observation.
μ = Overall mean.
Ti = Effect of the experimental treatments.
eij = The experimental error.

RESULTS AND DISCUSSION

Initial body weight, body weight after 8 weeks, final body weight , body weight gain and % changes in body weight at the end of the study were summarized in Table (1). It was observed that body weight increased significantly after 8 weeks in all groups but percentage of change in body weight in high fat diet groups (17.78%) were twice the percentage of normal diet groups (8.88%). After introducing mix solution, the percentage of change in body weight for high fat diet group (1.8%) were nearly as control normal diet group (2%). Dietary factors play a key role in the development of various human diseases [41]. It has also been indicated that the benefits of vegetable and fruit intake appear to be related to their phytochemicals contents which can demonstrate anti-inflammatory properties [42]. Many human and animal studies have investigated the effects of garlic on lipid levels, fasting blood sugar, BMI. The result of this study found that natural mix solution can decrease body weight significantly ($P<0.05$) for overweight rats how fed on high fat diet. The percentage of change in body weight gain in high fat diet groups were nearly as control group by using bioactive mixture LOG.

Relative Organs Weight: Table (2) describe the relative weight for organs in treatment groups it was noticed that there were insignificant changes in relative organs weight among all groups.

Hematological Parameters: Blood picture that presented in Table (3) cleared a significantly decreasing in RBC in Mixture groups (normal diet and high fat diet) (5.9×10^6 L) than control groups (normal diet and high fat diet)

Table 1: Body weight changes and body weight gain for different experimental group rats

	Experimental group rats			
Item	Control (ve-) (G_1)	Control (ve+) (G_2)	Mix ND (G_3)	Mix HFD (G_4)
No of rats in each group	10	10	10	10
Initial weight (IW), g	202.6±6.0	210.3±2.7	201.2±3.0	213.5±6.5
Duration period		8 weeks		
BW after 8 WKS (g)	220.6±8.0^{b}	247.7±5.2^{ab}	218.7±7.2^{ab}	251.4±6.4^{a}
BWG (g) after 8 WKS	18.0 ±2.0	37.4±2.5	17.5±4.2	37.9±0.1
% change after 8 WKS	8.88%	17.78%	8.7%	17.8%
FW (g)	225.0±7.2^{b}	256.5±5.6^{a}	229.0±11.3^{a}	255.8±8.1^{a}
BWG(g)	4.4±6.3^{b}	8.8±6.5^{a}	10.3±7.1^{a}	4.4±1.7^{ab}
% change of BW	2%	3.6%	4.7%	1.8%

Data are expressed as mean ± SE (N = 10).

a and b: Means in the same row having different superscripts differ significantly at level (P<0.05).

Means which share the same superscript are not significantly different; significance level at (P< 0.05).

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Mix ND: Normal diet + 1 ml LOG /h/day.

Mix HFD: High fat diet + 1 ml LOG /h/day.

Table 2: Relative organs weight for control groups and mix groups

	Experimental group rats			
Relative organs weight (%)	Control (ve-) (G_1)	Control (ve+) (G_2)	Mix ND (G_3)	Mix HFD (G_4)
Liver	3.5±0.1	2.9±0.1	3.3±0.2	2.8±0.3
Kidney	0.8±0.02	0.7±0.03	0.7±0.02	0.7±0.04
Heart	0.4±0.02	0.3±0.02	0.4±0.02	0.3±0.03
Lungs	0.9±0.04	0.9±0.04	0.8±0.02	0.8±0.06
Brain	0.7±0.02^{a}	0.7±0.02^{ab}	0.8±0.03^{a}	0.7±0.04^{a}
Spleen	0.4±0.02	0.3±0.02	0.4±0.01	0.3±0.05
Pancreas	0.34±0.01	0.4±0.03	0.4±0.01	0.3±0.03

Data are expressed as mean ± SE (N = 10).

a and b: Means in the same row having different superscripts differ significantly at level (P<0.05).

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Mix ND: Normal diet + 1 ml LOG /h/day.

Mix HFD: High fat diet + 1 ml LOG /h/day.

Table 3: Hematological parameters for control groups and mix groups

	Experimental group rats			
Parameters	Control (ve-) (G_1)	Control (ve+) (G_2)	Mix ND (G_3)	Mix HFD (G_4)
RBC ($\times 10^6$ L)	7.0 ±0.1^{a}	7.1±0.1^{a}	5.9±0.3^{b}	5.9±0.3^{b}
WBC ($\times 10^3$ L)	20.6±1.5^{a}	10.9±0.4^{b}	18.04±0.8^{a}	9.6±0.2^{c}
Hemoglobin (g/dl)	15.6±0.2^{c}	17.8±0.1^{a}	16.6±0.3^{b}	15.4±0.4^{c}
Platelets ($\times 10^3$ L)	716.3±17.4^{a}	576±4.5^{b}	535.2±9.1^{b}	500.8±6.9^{c}
HT (g/dl)	46.6±0.7^{b}	53.30±0.2^{a}	46.9±1.4^{b}	45.7±1.2^{b}

Data are expressed as mean ± SE (N = 10).

a, b and c: Means in the same row having different superscripts differ significantly at level (P<0.05).

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Mix ND: Normal diet + 1 ml LOG /h/day.

Mix HFD: High fat diet + 1 ml LOG /h/day

(7, 7.1 x 10^6 L). It was also, found that WBC were significantly decrease in Mixture high fat group (9.6 x10^3 L) than control ve-group (20.6 x10^3 L), control ve+ group (10.9 x10^3 l) and Mixture normal diet group (18.04 x1 0^3 L).Hemoglobin level was significantly higher in control ve+ group 17.8 g/dl) than other treatment group but all group were in normal level. For platelets it was found that mixture high fat diet group recorded the significant lowest count of platelets (500.8 x10^3 L) than other groups. Natural mixture also significantly (P<0.05) reduced WBC in high fat diet group than control group, but there wasn't any significant effect on other hematological parameters.

Table 4: Kidney functions for control groups and mix groups

	Experimental group rats			
Parameters	Control (ve-) (G_1)	Control (ve+) (G_2)	Mix ND (G_3)	Mix HFD (G_4)
Uric acid (mg/dl)	9.3 ± 0.2[a]	2. 7± 0.1[c]	6.9±0.4[b]	3.2±0.2[c]
Creatinine (mg/dl)	0.58 ± 0.02[ab]	0.64 ± 0.04[a]	0.56±0.02a[b]	0.56±0.02a[b]
Urea (mg/dl)	56.7 ± 0.8[b]	72.0 ± 2.7[a]	57.4±1.2[b]	59.7±2.2[b]

Data are expressed as mean ± SE (N = 10).

a, b and c: Means in the same row having different superscripts differ significantly at level (P<0.05).

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Mix ND: Normal diet + 1 ml LOG /h/day.

Mix HFD: High fat diet + 1 ml LOG /h/day.

Table 5: Lipid profile for control groups and natural mix groups

	Experimental group rats			
Parameters	Control (ve-) (G_1)	Control (ve+) (G_2)	Mix ND (G_3)	Mix HFD (G_4)
TG (mg/dl)	146.3 ± 4.4[b]	172.7 ± 2.7[a]	96.5±10.5[c]	94.2±1.9[c]
CHOL (mg/dl)	123.1 ± 1.4[b]	130.4 ± 2.8[a]	99.4±1.1[c]	99.2±3.9[c]
HDL (mg/dl)	56.4 ± 1.0[b]	46.4 ± 2.5[b]	64.7±1.5[a]	54.4±2.4[b]
LDL (mg/dl)	37.4± 1.2[a]	35.5± 1.8[a]	15.4± 1.4[b]	5.6± 2.5[c]
VLDL (mg/dl)	29.3 ± 0.9[b]	48.5 ± 0.5[b]	19.3±2.1[a]	39.2±0.2[c]

Data are expressed as mean ± SE (N = 10).

a, b and c: Means in the same row having different superscripts differ significantly at level (P<0.05).

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Mix ND: Normal diet + 1 ml LOG /h/day.

Mix HFD: High fat diet + 1 ml LOG /h/day.

Kidney Function: Kidney function for control groups fed on normal diet, high fat diet and treatment groups were describe in Table (4). It was found that mix causes significant decrease in the level of uric acid in normal diet group (6.9 mg/dl) than control group (9.3 mg/dl) but there was in significant change between mix high fat diet (3.2 mg/dl) and control high fat diet (2.7 mg/dl). For creatinine it was noticed slight decrease in mix normal diet group (0.56 mg/dl) than control normal diet group (0.58 mg/dl), while it was found significant decrease in creatinine level in mix high fat diet (0.52 mg/dl) than control high fat diet (0.64 mg/dl). Urea level was significantly decrease in mix high fat diet (59.7 mg/dl) than control high fat diet (72 mg/dl), while it was in significant change in urea level between mix normal diet and control normal diet.

The present results of kidney function were in agreement with those obtained by Omer *et al.* [43] who reported that adding LOG in rabbit diets significantly (P<0.05) decreased creatinine; however, rabbits receiving diet containing 2% LOG significantly (P< 0.05) decreased alkaline phosphatase compared to the control group and rabbits that received 0.5% or 1% LOG. In addition to, in another study on laying hen, Omer *et al*. [44] mentioned that adding LOG at different levels insignificantly decreased blood plasma content of creatinine in comparison with the control.

Lipid Profile: As shown 1n Table (5) incorporation the mixture of LOG caused significantly decreasing in triglycerides in both normal diet and high fat diet comparing to the control groups. The level of tri glyceride was (146.3 and172.7 mg/dl) in control ve- and control ve+ respectively while it became 96.5 and 94.2 mg/dl in mixture normal diet and mixture high fat diet. Also, for cholesterol it was noticed that using mixture solution caused significantly decreasing in cholesterol level in mixture normal diet group (59.4mg/dl) and mixture high fat group (59.2 mg/dl) than control ve- group (83.1 mg/dl) and control ve+ group (90.4 mg/dl). HDL level increased significantly by using mixture ether in normal diet group (64.7 mg/dl) or in high fat diet group (54.4 mg/dl) than control ve- group (56.4 mg/dl) and control ve+ group (46.4 mg/dl).

It was noticed that high fat diet caused significant increasing in triglyceride, cholesterol and VLDL than normal diet, but using mixture solution LOG can reduce the level of triglyceride, cholesterol and VLDL significantly. Than control normal diet and control high fat diet. The results of other studies showed that combination of garlic and lemon juice significantly reduced serum TC, LDL-C and blood pressure. Garlic, such as many other food additives, gained substantial interest due to its effects on lipid levels [45-47]. Also, Saffa [48] noticed a reduction in total

concentration of plasma cholesterol as well as LDL by adding garlic and fenugreek to the diet of laying hens. Ao *et al.* [49] studied the effect of using garlic powder on plasma cholesterol concentration of laying hens and they noted that by increasing the amount of garlic powder in the diet from 0.00 to 3%, cholesterol level has been significantly decreased. Meanwhile, Saki *et al.* [50] observed that levels of serum HDL in laying hen fed with diets containing 8 or 12 g kg^{-1} herb mixture were increased significantly. Interestingly, LDL levels were increased by supplementation of phytogenic feed additive treatments. However, serum triglyceride and cholesterol concentration were not affected by herb mixture supplementation. Omer *et al.* [43] noted that incorporation of LOG as natural bioactive mixture in rabbit rations at 1.5% or 2% significantly (P<0.05) decreased total cholesterol in comparison with the control, while the addition of 0.5% or 1% LOG did not alter significantly with the other groups. Also, they reported that treatments contained (1%, 1.5% and 2% LOG) significantly (P<0.05) decreased low-density lipoprotein (LDL), while it had no significant effect on high-density lipoprotein (HDL) for rabbits. In addition, Hassan and Abdel-Raheem [51] found that calves fed with diets containing garlic caused lowering in cholesterol content compared to those fed with the control diet. Different extracts of garlic alone have been demonstrated to lower serum cholesterol, triglycerides and LDL in rodents and humans [52, 53]. Also, Maha and Khalil [54] showed that adding 8% raw garlic along with 2% cholesterol to the diet of rats decreased plasma TC and LDL-C.

Liver Enzymes: Liver enzymes that listed in Table (6) cleared that mixture caused significant decrease in ALT in normal diet group (39.7 mg/dl) and high fat diet group (35.2 mg/dl) than control ve- group (44.5 mg/dl) and control ve+ group (56.2 mg/dl). For AST it was found that in mix normal diet group mix keep the level of AST as normal control normal diet (ve-), also, it caused slight decrease in mix high fat diet (37.3 mg/dl) than control high fat diet (ve+) (41.5 mg/dl). Liver enzymes as indicators for liver functions were significantly affected by adding high fat. High fat diet caused significant increase in ALT and AST but it caused significant decrease in ALP, but using mix solution enhances liver enzymes than control groups. Omer *et al*. [44] also found that Inclusion of LOG at different levels significantly (P<0.05) decreased blood plasma contents of GPT and GOT in laying hens.

Table 6: Liver enzymes for control groups and natural mix groups

	Experimental group rats			
Parameters	Control (ve-) (G_1)	Control (ve+) (G_2)	Mix ND (G_3)	Mix HFD (G_4)
ALT	44.5 ± 1.4^{c}	56.2±1.4^{a}	39.7±1.6^{b}	35.2±1.7^{d}
AST	24.7 ± 0.8^{b}	41.5±0.8^{a}	24.9±0.6^{b}	37.3±0.9^{a}
ALP	296.8 ± 4.4^{a}	41.2 ±1.2^{b}	282.2±10.8^{a}	41.8±0.9^{b}

Data are expressed as mean ± SE (N = 10).

a, b, c and d: Means in the same row having different superscripts differ significantly at level (P<0.05).

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Mix ND: Normal diet + 1 ml LOG /h/day.

Mix HFD: High fat diet + 1 ml LOG /h/day.

Table 7: Biochemical parameters for control groups and natural mix groups

	Experimental group rats			
Parameters	Control (ve-) (G_1)	Control (ve+) (G_2)	Mix ND (G_3)	Mix HFD (G_4)
Total protein	90.9 ± 2.3^{b}	88.1 ± .6^{b}	104.6±2.5^{a}	89.0±1.8^{b}
Albumin	48.4 ± 0.4^{a}	35.2 ± 1.6^{b}	46.4±1.2^{a}	33.8±1.1^{b}
Globulin	42.5 ± 2.5^{c}	52.9 ± 2.2^{ab}	48.3±1.9^{bc}	55.2±1.8^{a}
T. Bilirubin	0.25 ± 0.02^{c}	0.66± 0.01^{a}	0.24±0.05^{c}	0.48±0.04^{b}
Glucose	120.0±4.3^{c}	139.9 ± 3.0^{a}	113.4±1.0^{c}	128.0±2.5^{b}

Data are expressed as mean ± SE (N = 10).

a, b and c: Means in the same row having different superscripts differ significantly at level (P<0.05).

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Mix ND: Normal diet + 1 ml LOG /h/day.

Mix HFD: High fat diet + 1 ml LOG /h/day.

Biochemical Parameters: Results of biochemical parameters that tabulated in Table (7) mentioned that mixture caused significantly increasing in T. Protein (104.6 mg/dl), Albumin (56.4 mg/dl) and T. Bilirubin (0.66 mg/dl) than control ve- group (90.9 mg/dl, 48.4 mg/dl and 0.25 mg/dl) respectively. For Albumin it was found that Mix caused insignificant decrease in albumin level (46.4 mg/dl and 33.8 mg/dl) in normal diet group and high fat group respectively than control ve- and ve+ groups (48.4 mg/dl and 35.2 mg/dl) respectively. Mix also caused insignificant increase in globulin level in both normal diet group (48.3 mg/dl) and high fat diet group (55.2 mg/dl) than control ve- (42.5 mg/dl) and control ve+ (52.9 mg/dl) respectively. T. Bilirubin was insignificantly decrease in mix normal diet group (0.24 mg/dl) than control ve- group (0.25 mg/dl), while it was significantly decrease in mix high fat diet (0.48 mg/dl) than control ve+ group (0.66 mg/dl). For Glucose it was found that mix caused significant decrease in glucose level in both normal diet group (113.4 mg/dl) and high fat diet group (128.0 mg/dl) than control ve- (120.0 mg/dl) and control ve+ group (139.9 mg/dl) respectively.

The present results recorded that; mixture insignificantly increase the level of total protein, Albumin and Globulin, this in agreement with those noted by Omer *et al.* [43] who indicated that the incorporation of LOG in growing rabbits at 0.5, 1, 1.5 and 2% had no significant effect on total protein and globulin, while it significantly affected ($P<0.05$) albumin for rabbit receiving 1 or 1.5% LOG and albumin to globulin ratio for rabbit offered with diet containing 1.5% LOG. Also, Ahmed *et al.* [55] reported that serum total protein was not significantly affected by the natural additive (LOG) when added to calves' rations. This indicates that the supplement had not affected protein synthesis in liver function. Also, the low level of proteins may be attributed to a decrease in the protein absorbed and synthesized and an increase in protein losses. Also, Hassan and Abdel-Raheem [51] mentioned that calves fed with diets containing garlic had greater ($P<0.05$) serum concentrations of globulin. T. Bilirubin was significantly decreased by using high fat diet than control high fat diet, also mix significantly reduce the level of glucose in both normal diet and high fat diet.

CONCLUSIONS

From the present study we can concluded that adding mix of LOG to overweight rats has the benefit effect on reducing body weight gain and can controlled the lipid contents in the body.

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