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Nutritional and Biochemical Studies of Annona (*Annona Squamosa*) and Lemon (*Citrus Aurantifolia*) Leaves Powder on Hyperlipidemic Rats

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Abstract

This study was carried out on the effect of nutrition and biochemical of Annona and lemon leaves powder on hyperlipidemic rats. The present study was designed to determine the effect of both Annona and lemon leaves powder on hyperlipidemic rats. Forty adult albino rats Sprague –Dawley strain weighing 120 ±10 g was randomly classified into eight groups. Five rats served as negative control group that fed on basal diet only while the other 35 rats were fed by10% animal fat. Rats were randomly into positive control group, six treated rat groups that were fed on 2.5%,5% and 7.5% of Annona and lemon leaves powder. The study period was 28 days. Phenolic compounds of Annona and lemon leaves were determined using HPLC technique. The results of the obtained data indicated that the treated groups (hyperlipidemic rats) resulted in decreased TC, TG, LDL& VLDL in the blood and increased HDL compared with positive control group with significant difference (P≤0.05). Also, the results indicated that the treated groups resulted increased oxidative enzymes (CAT, SOD& GSH) compared with positive control group with significant difference (P≤0.05). Also, the obtained results indicated that the treated groups showed decreased in (AST, ALT& ALP) compared with positive control group with significant difference (P≤0.05). The result of phenolic compounds of Annona and lemon leaves revealed that each leaves contains high amounts of phenolic compounds, and it can use as antioxidant. The study clearly demonstrated that rats fed on Annona and lemon leaves powder could ameliorate the hyperlipidemia in rats.

Key words*: Plant leaves, Rats, Biochemical analysis, Hyperlipidemia.*

Introduction

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases stroke, atherosclerosis and hyperlipidemia are the primary cause of death. Hyperlipidemia is characterized by elevated serum total cholesterol (TC), low density lipoprotein (LDL), and very low-density lipoprotein (VLDL) and decreased high density lipoprotein (HDL) levels. Among these, hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (Jackson and Beaglehole., 1995). The prevalence of elevated total cholesterol was highest in the WHO Region of Europe (54% for both sexes), followed by the WHO Region of the Americas (48% for both sexes). The WHO African Region and the WHO Southeast Asian Region showed the lowest percentages (22.6% and 29.0%) (WHO, 2008). Growing Population: The country has an estimated population of 91.5 million in 2015, which is expected to reach151 million by 2050.Egypt had around 24 million people over the age of 40 in 2015 Hyperlipidemia: 38.6% of the population suffer from raised cholesterol levels (Capmasd and Colliers, 2017). Medicinal plants are beneficial to the human body as they are used for medicinal purposes or as dietary supplements. Plants have been the basis of medicinal treatment since prehistoric times, and herbal remedies are still widely practiced today. Modern medicine makes use of many compounds derived from plants as an essential raw material in the pharmaceutical industry (Benzie and Wachtel-Galor , 2011). Annona squamosa leaves and Citrus aurantifolia leaves which were contains several medicinal properties and Antioxidants. Therefore, it was used in this study as a tool to treat the problem of hyperlipidemia. Annona squamosa leaves which contains Antioxidants are the compounds responsible for the protection of living organism from the damage caused by the abnormal production of reactive oxygen species concomitant lipid peroxidation, protein damages and others including DNA strand breaking (the enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione. Which it helps Anona squamosa significantly reduced triglyceride and total cholesterol levels with a gradual increase in HDL cholesterol in lab mice and helps improve fat metabolism (Kumar, et al., 2008). Citrus aurantifolia leaves contains active phytochemical substances as follows: flavonoids, phenols, and limonoids. phytochemical properties of C. aurantifolia from several literature reviews are described as antibacterial, anti-inflammation anti-lipidemia, antioxidant and antiparasitic, it is used for the treatment of cardiovascular, hepatic and urolithiasis diseases. Moreover, it can be used for insecticide activity (Ezekwesili-Ofili, and Gwacham.,2015).

Based on all above, the aim of this investigation which concentrated on study the effect of nutrition and biochemical of Annona and lemon leaves powder on female hyperlipidemic rats.

Materials And Methods

Materials

Annona (Annona squamosa) and lemon (Citrus aurantifolia) leaves were obtained from Faculty of Agriculture Menoufia university, since 2019 at Shebin El Kom. A total of 40 adult normal albino rats Sprague Dawley strain weighting 120 ±10 g were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt. Rats were in cages under the normal laboratory condition and were fed on standard diet for seven days as an adaptation period. Diet was offered to rats in special feed cups to avoid looser conditions of feed, water was provided to the rats by glass tubes supported to one side of the cage, food and water provided labium and checked daily. Casein, cellulose, choline chloride and DL methionine powder were obtained from Morgan Co. Cairo, Egypt. All chemical kits used in this study El-Gomhoria Company for Trading Chemical, Drugs and Medical Instruments, Cairo, Egypt. While the animal fat obtained from a butcher shop.

Preparation of Annona and lemon leaves

After collecting Annona and lemon leaves, the plant was washed thoroughly under running tap water. Where dried at 40°C in vacuum oven. The dried leaves were ground in a grinder (Braun Biotech International GMBH.D.34212 Melsungen, Germany) to pass through a 1.6 mm sieves and stored at -12°C until used.

Experimental animals

Forty albino rats, Sprague Dawley Strain, weight 120 ± 10g. The animals were classified in to 8 groups it consists of five rats from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt. Experimental was applied in animal Experimental laboratory of the Faculty of Home Economics, Menoufia University Rats were kept in cages wire. The diet was introduction in special feed cups to avoid scattering of feed also water was provided to the rats by glass tube throughout the wire case.

The induction of experimental hyperlipidemia

Hyperlipidemia was induced in normal healthy albino rats by 10% animal fat for 28 days.

Experimental design

All groups of rats were fed on the experimental diet for 28 days according to the following groups: Rats were randomly divided into two main groups. The first group, negative control group (n=5), Fed standard diet only. The second main group hyperlipidemic rats (n=35). Hyperlipidemic rats were divided into 7 groups according to the following groups: Group (1): Positive control, fed standard diet only. Group (2): Fed standard diet replaced with 2.5% of Annona leaves powder. Group (3): Fed standard diet replaced with 5% of Annona leaves powder. Group (4): Fed standard diet replaced with 7.5% of Annona leaves powder. Group (5): Fed standard diet replaced with 2.5% of lemon leaves powder.

Group (6): Fed standard diet replaced with 5% of lemon leaves powder. Group (7): Fed standard diet replaced with 7.5% of lemon leaves powder.

The basal diet in the experiment consisted of casein (10%), corn oil (10%) vitamin mixture (1%), salt mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%) and the remained is corn starch (69.5%) according to (Campbell, 1961). At the end of the experimental (4 weeks), Blood samples were collected after 12 hours fasting at the end of the experiment. Using the retro-orbital method by means of a micro capillary glass tubes, blood was collected into a dry clean centrifugal tube and left to clot in a water bath (37°C) at room temperature for half an hour. The blood was centrifuged for 10 minutes at 3000 rpm to separate the serum in clean glass well stoppered and stored at and kept (-20°C) until analysis (Schermer, 1967).

Determination of serum lipids

Total cholesterol (TC), Triglycerides (TG), HDL-cholesterol, VLDL and LDL-cholesterol were determined according to the methods described by (Allen, 1974), (Fassati and Prencipe 1982), (Lopez, 1977) and (Lee and Nieman, 1996)respectively.

Determination of liver functions

Alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP) were determined according to the methods described by (Clinica Chimica Acta,(1980), Hafkenscheid (1979) and Moss (1982) respectively.

Determination of oxidative enzymes

Catalase Enzyme (CAT) UL/ml, Superoxide dismutase (SOD) U/ml and Glutathione (GSH) UL/ml were determined according to the methods described by Jimmy (1994), Jentzsch et al ., (1996) and Aebi (1974) respectively.

Determination of phenolic compounds:

HPLC analysis of extracts was performed using an Agilent 1200 chromatograph equipped with a PDA model G1315B, a Bin pump odel G1312A, an auto-sampler model G1313A and a RR Zorbax Eclipse Plus C18 column (1.8 µm, 150 mm ×4.6 mm). The mobile phase A was 0.2 % formic acid in water and the mobile phase B was acetonitrile. The HPLC method was used according to Rajkumar et al., (2011).

Statistical Analysis

Data were analyzed using a completely randomized design (SPSS, 2010) when a significant main effect was detected, the means were separated with the student-Newman-Keuls test. Differences between treatments of ($P \leq 0.05$) were considered significant (Wolfinger and Chang, (1995).

Results and Discussion

Data given in Table (1) show effect of Annona and lemon leaves powder on serum lipid profile of hyperlipidemic rats. The obtained results indicated that the highest (TC) , (TG),

(LDL) and (VLDL) recorded for positive control group, while the lowest level recorded for negative control group with significant differences (P≤0.05) (227.55±12.38 ,109.78±5.25 &180.70±3.11 , 89 .40±0.96 & 154.03±8.07 , 29.86±2.74 & 35.14±0.64 and 17.92±0.19) mg\dl respectively, the highest HDL level recorded for 7.5% Annona and lemon leaves (53.46±1.94& 55.81±7.63) mg/dl, respectively. while the lowest HDL level recorded for 2.5% Annona and lemon leaves (49.55±1.64& 48.38±4.36) mg/dl, respectively. The better serum((TC), (TG), (HDL), (LDL) & (VLDL) was showed for hyperlipidemic rats fed on 7.5% Annona leaves and 7.5% lemon leaves) when compared to control (+) group. The value was (145.48±9.24, 173.78 ± 1.72&134 .10±4.72 , 138.00±3.82 &53.46±1.94, 55.81 ± 7.63&78 .22±4.97 , 78.09 ± 26.61 &13.80±0.57, 30.88±20.58) mg/dl, respectively .these results were in accordance with (Rajesh et al ., (2005) they reported that Annona leaves was administered orally It brought about fall in the level of (TC) by with increase of (HDL) and decrease of (LDL) , (VLDL) and (TG) levels, respectively. This result is agreeing with also (Lin et al., 2019) where revealed lemon leaves increased HDL in rats fed on high fat diet and decrease of (TG), (LDL) and (VLDL) levels.

Table (1): Effect of Annona and lemon leaves on serum lipids of hyperlipidemic rats

Categories	Control (-)	Control (+)	Annona Leaves			Lemon Leaves		
			2.5%	5%	7.5%	2.5%	5%	7.5%
Cholesterol (g)%	109.8^{e}±5.3	227.5^{a}±12.3	215.4 ^{a} ±2.6	194.3 ^{a}±6.1	145.4^{d} ±9.2	221.2^{a} ±6.9	198.1 ^{a}±4.7	173 .8^{b} ±1.7
Triglycerides (TG) mg\dl	89 .4^{e} ±0.9	180.7^{a}±3.1	170 .1^{a} ±6.7	156 .0^{b} ±2.8	134 .1^{c} ±4.7	175.4^{a} ±2.0	157.3^{b} ±1.4	138 .0^{c} ±3.8
HDL mg\dl	62.0^{a}±3.6	38.3^{d}±5.3	49.5^{c} ±1.6	52.1^{b} ±4.1	53.4^{b} ±1.9	48.4^{c} ±4.3	50.5^{b} ±4.1	55.8^{b} ±7.6
(LDL) mg\dl	29.86^{e}±2.7	154.0^{a}±8.0	132.7^{b} ±3.7	119.8^{c} ±8.4	78.2^{d} ±4.9	134.0^{b} ±8.5	111.7^{c} ±6.6	78.1^{d} ±26.6
(VLDL) mg\dl	17.9^{e}±0.1	35.1^{b}±0.6	33.1^{b} ±0.7	22.4^{d} ±0.9	13.8^{f} ±0.5	38.8^{a} ±0.2	36.0^{a} ±0.7	30.8^{c} ±20.5

Data given in Table (2) show effect of Annona and lemon leaves on serum lipids (AST, ALT and ALP) of hyperlipidemic rats. The obtained results indicated that the highest AST, ALT and ALP recorded for positive control group, while the lowest level recorded for negative control group with significant differences (P≤0.05) being (97.25±5.82, 38.06±1.07 & 86.10±1.10, 39.51±0.94 and 96.19±6.10 40.81±3.04) U/L, respectively. On the other hand, rats fed on 2.5% Annona and lemon leaves was the highest AST, ALT and ALP level recorded with significant differences (P≤0.05) being 77.52±7.22,

88.66±1.76& 85.93±1.25, 71.79±2.28 and 75.73±9.25, 91.29±4.06 U/L, respectively. The better serum (AST), (ALT) and (ALP) was showed for hyperlipidemic rats fed on 7.5% Annona leaves and 7.5% lemon leaves 7.5% when compared to control (+) group. The value was (56.12±0.51, 74.87±0.42&62.80±0.48, 60.44±0.79 and 52.90±6.88, 73.94±5.09) U/L, respectively. these results were in accordance with this study agree with Mohamed, (2008) who explained that aqueous and alcoholic extract of leaves of Annona were used for the screening of hepatoprotective activity significant decrease in (AST, ALT and ALP) in treatment group as compared to control group. It should be concluded that the extracts of Annona It have a role in treating the liver. This study agree with also Affiong et al., (2016)) the effects of extract of lemon on some blood and liver parameters were examined. Graded of extracts were administered daily to rats for 14 days and the effects on body weight, liver enzymes and some blood parameters were evaluated. the results showed significant decreases in alanine amino transferase (AST), (ALT) and (ALP) when compared to the control group. it should be concluded that the extracts of lemon It has a role in treating the liver.

Table (2): Effect of Annona and lemon leaves on liver enzyme (AST, ALT and ALP) of hyperlipidemic rats

Categories	Control (-)	Control (+)	Annonasquamosa leaves 2.5%	5%	7.5%	Citrus aurantifolia leaves 2.5%	5%	7.5%
(AST) mg\dl	38.06f± 1.07	97.25a± 5.82	77.52c± 7.22	65.10d± 4.92	56.12e±0 .51	88.66b± 1.76	74.87c± 0.42	62.31d± 1.80
(ALT) mg\dl	39.51d± 0.94	86.10a± 1.10	85.93a± 1.25	74.43b± 1.21	62.80c±0 .48	81.29a± 0.26	71.79b± 2.28	60.44c± 0.79
(ALP) mg\dl	40.81f± 3.04	96.19a± 6.1	75.73c± 9.25	64.03d± 6.21	52.90e±6 .88	91.29a± 4.06	81.79b± 7.88	73.94c± 5.09

Values are expressed as means ± SD; means in the same row with different letter are significantly different (P < 0.05).

Data given in Table (3) show the effect of Annona and lemon leaves on oxidative enzyme (CAT, SOD and GSH) of hyperlipidemic rats. The obtained results indicated that the highest (CAT), (SOD) and (GSH) recorded for negative control group, while the lowest level recorded for positive control group with significant differences (P≤0.05 being (65.4±3.11, 18.18±4.65& 36.22±5.01, 9.98±3.4265& and 79.15±3.11 , 21.58±1.92) IU/ml respectively. On the other hand, rats fed on 2.5% Annona and lemon leaves was the lowest (CAT), (SOD) and (GSH) level recorded with significant differences (P≤0.05) being (31.71±4.05, 25.14±3.99& 18.51±4.55, 15.84±4.39 and 22.91±3.05, 21.04±3.09) IU/ml, respectively. The better serum (CAT), (SOD) and (GSH) was showed for hyperlipidemic rats fed on 7.5% Annona leaves and 7.5% leaves when compared to

control (+) group. The value was (54.60±7.72, 54.12±5.75 & 33.20±6.12, 33.52±3.75 and 66.90±2.02, 64.67±1.95) IU/ ml, respectively. This study agrees with (Pélissler et al ., 1994 & Pandey and Dushyant ., 2011 who explained that leaves of Annona species exhibit antioxidant activity in different in vitro models due to the presence of (CAT), (GSH), and (SOD). Many studies prove that every part of A. squamosa possess medicinal property Sandra et al., (2012). Lemon leaves which content antioxidant enzymes reduce blood lipids such as SOD, CAT and GSH Somaieh et al .,(2011).

Table (3): Effect of Annona and lemon leaves on the oxidative enzymes of hyperlipidemic rats

Categories	Control (-)	control (+)	Annona leaves			Lemon leaves		
			2.5%	5%	7.5%	2.5%	5%	7.5%
(CAT)	$65.4^{a}\pm$	$18.18^{f}\pm$	$31.71^{d}\pm$	$41.73^{c}\pm$	$54.60^{b}\pm$	$25.14^{e}\pm$	$39.23^{c}\pm$	$54.12^{b}\pm$
IU/ml	3.11	4.65	4.05	6.40	7.72	3.99	5.81	5.75
(SOD)	$36.22^{a}\pm5.$	$9.98^{d}\pm3$	$18.51^{c}\pm$	$22.07^{b}\pm$	$33.20^{a}\pm$	$15.84^{c}\pm$	$22.03^{b}\pm$	$33.52^{a}\pm$
IU/ml	01	.42	4.55	4.70	6.12	4.39	3.31	3.75
(GSH)	$79.15^{e}\pm3.$	$21.58^{a}\pm$	$22.91^{a}\pm$	$34.42^{c}\pm$	$66.90^{d}\pm$	$21.04^{a}\pm$	$32.53^{c}\pm$	$64.67^{d}\pm$
IU/ml	11	1.92	3.05	2.90	2.02	3.09	2.91	1.95

Values are expressed as means ± SD; means in the same row with different letter are significantly different (P ≤ 0.05).

Table(4):Identified of phenolics compounds in Annona and lemon leaves by HPLC

Phenolic compounds	Annona(Annonasquamosa) leaves Concentration (mg/100g)	Lemon(*Citrus aurantifolia)* leaves Concentration (mg/100g)
Gallic acid	0.48	37.20
P- coumaric acid	0.10	-
Chlorogenic acid	0.65	-
Vanillin	0.34	-
Ferulic acid	0.18	1340
Syringic acid	0.014	136.50
Hydroxybenzoic acid	0.89	200.10
Caffeic acid	ND	570.25
Cinamic acid	ND	126.10
P- coumaric acChlorogenic acid	-	61.45
Vanilic acid		68.20
Epicatechin	-	200.10
Caffeic acid	-	200.70
Pyrogallol	-	2150
Catechol	-	
	-	
LSD	1.45	1.45

ND= Not detected

The highest values of phenolic compounds in the leaves of Annona were recorded hydroxy benzoic acid. The lowest values of phenolic compounds in the leaves of Annona

were recorded syringic acid. The highest values of phenolic compounds in the leaves of lemon were recorded catechol. The lowest values of phenolic compounds in the leaves of lemon were recorded gallic acid. Phenolic compounds exist in most plant tissues as secondary metabolites but may play roles as antioxidants reduce blood lipids according Shahriar and Robin (2010).

Conclusion

Used of Annona leaves and lemon leaves powder improved the level of hyperlipidemia, due to it contains many phenolic compounds can use as antioxidant. Therefore, the data recommended Annona and lemon leaves selected in a moderate amount to be included in our daily diets.

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دراسات تغذوية وكيموحيوية لتأثير مسحوق أوراق القشطة والليمون على الفئران البيضاء المصابة بارتفاع دهون الدم

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أجريت الدراسة لمعرفة التأثيرات التغذوية والكيميائية الحيوية لمسحوق القشطة ومسحوق أوراق الليمون على إناث الفئران المصابة بارتفاع دهون الدم. صممت الدراسة الحالية لتحديد تأثير مسحوق أوراق القشطة والليمون على إناث الفئران المصابة بارتفاع دهون الدم .تم استخدام أربعين أنثى بالغة من الفئران البيضاء من سلالة سبراجيو ديولى التي يبلغ وزنها ١٢٠ ± ١٠ جم بشكل عشوائي إلى ثماني مجموعات .تم استخدام خمسة فئران كمجموعة ضابطة سالبة والتي تغذت على النظام الغذائي الأساسي فقط بينما تم تغذية الخمسة والثلاثين الفئران الأخرى بنسبة 10 ٪ من الدهون الحيوانية .تم وضع الفئران عشوائياً في المجموعة الضابطة الموجبة، ستة مجموعات من الفئران تمت تغذيتها على 2,5 ٪، ٥٪، 7,5 ٪ من مسحوق أوراق القشطة والليمون .كانت فترة الدراسة ٢٨ يومًا .كذلك تم التعرف على المركبات الفينولية في أوراق مسحوق القشطة والليمون باستخدام جهاز الكروماتوجرافى السائل عالي الأداء. أشارت النتائج التي تم الحصول عليها إلى أن المجموعات المعالجة (الفئران المصابة بارتفاع دهون الدم) أدت إلى انخفاض في قيم كلا من الجلسريدات الثلاثية، الكوليسترول الكلى والكوليسترول منخفض الكثافة و الكوليسترول منخفض الكثافة جدا في الدم وزيادة مستوى الكولسترول عالي الكثافة مقارنة مع المجموعة الضابطة الموجبة مع وجود فرق معنوي (P≤0.05) .كما أشارت نتائج الدراسة التي تم الحصول عليها إلى أن المجموعات المعالجة أدت إلى زيادة إنزيمات الأكسدةCAT) ، (SOD & GSHمقارنة بالمجموعة الضابطة الموجبة مع وجود فرق معنوي(P≤0.05) . كما أوضحت النتائج المتحصل عليها أن المجموعات المعالجة أظهرت انخفاضاً في انزيمات الكبد (ALP ، ALT، AST) مقارنة مع المجموعة الضابطة الموجبة مع اختلاف معنوي(P≤0.05) . اظهرت نتائج المركبات الفينولية أن أوراق مسحوق القشطة والليمون تحتوى على العديد من المركبات الفينولية والتي يمكن ان تستخدم كمضادات أكسدة. الخلاصة: أظهرت الدراسة بوضوح أن الفئران التي تغذت على مسحوق القشطة ومسحوق أوراق الليمون يمكن أن تحسن الحالة الصحية المصابة بارتفاع دهون الدم في الفئران.

الكلمات المفتاحية: أوراق النباتات . الفئران . التحاليل الكيميائية الحيوية . ارتفاع دهون الدم.