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IMPACT OF GUAVA AND MANGO LEAVES ON HYPERLIPIDEMIC ALBINO RATS

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ABSTRACT: The present study was designed to investigate the chemical composition of guava (*Psidium guajava*) and mango (*Mangifera indica*) leaves and evaluate their potential effect as hpolipidemic agents in experimental animals. Twenty five male albino rats were divided into five groups (group A, represent control; group B, was received high fat diet to serve as positive control, group C, feeding on diet contain 10% mango leaves, group D, feeding on diet contain 10% guava leaves and group E, feeding on diet contain 5% guava and 5% mango leaves. Proximate analysis of guava and mango leaves showed that they contain: total carbohydrates (70.66% and 75.53%, respectively), crude protein (22.78% and 18.45%, respectively), ash (5.47% and 4.82%, respectively) and crude fat (1.09% and 1.2%, respectively). Total phenolic compounds in guava and mango leaves were 118.1 and 73.7 mg/g, respectively, while, total flavonoids were 11 and 9.75 mg/g, respectively. Fourteen phenolic compounds were identified from the results of the HPLC analysis of the methanolic extract of leaves of both guava and mango. The most important compounds in guava leaf extract were gallic acid, catechin, and chlorogenic acid, while catechin, Pyro catechol, and gallic acid were the most important compounds in the alcoholic extract of mango leaves. The most important results of the biological experiment indicated that group B was the worst of all measures of levels of bad fats in the blood (triglycerides, total cholesterol and LDL-cholesterol). Both guava and mango leaves (groups C and D) led to a significant decrease in the levels of triglycerides, total cholesterol and LDL-cholesterol, while group E (mix of guava and mango leaves) showed the best results in general. In a conclusion, both guava and mango leaves can be used in diets to lower blood fats.

Key words: Fruit leaves, Rats, High fat diet, Phenolic compounds.

INTRODUCTION

Abnormal lipid metabolism is a main cause of dyslipidemia, which is a major risk factor for cardiovascular disease, obesity and overall mortality (Rizvi *et al.*, 2003). Blood cholesterol is of great importance because blood total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) correlate strongly with coronary heart disease (CHD). The concentration of plasma cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from the circulation, absorption of dietary cholesterol and excretion of cholesterol via bile and feces (Choi *et al.*, 2001). Many medicinal plants have been used in various traditional systems, for lipid management. Many kinds of Hypocholesterolemic activities from a lot of plant materials were confirmed till now (Chi *et al.*, 1998).

The term, medicinal plants, includes various types of plants have medicinal activities. These medicinal plants are considered as a rich resource of ingredients which can be used in drug development and synthesis (Hassan, 2012). Herbal medicine is more accessible to most of the population. About 60 to 85% of the populations of every country of the developing world rely on herbal or indigenous forms of medicine (Onyeka *et al.*, 2012). Mango *(Mangifera indica)* has therapeutic potential in treating obesity and related diseases through regulating the expression of transcriptional factors and enzymes associated with adipogenesis (Ramírez *et al.*, 2017). Medicinal properties of guava (*Psidium guajava)* fruit, leaf and other parts of the plant are well known in traditional systems of medicine. Phytochemical compounds such as carotenoids and polyphenols, the major classes of antioxidant pigments give

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guava a relatively high potential antioxidant value among plant foods (Joseph and Priya, 2011). Guava leaves extract are also widely used for their antispasmodic, cough sedative, anti-inflammatory, antidiarrheic, antihypertension, antiobesity, and antidiabetic properties (Chen and Yen 2007). The aim of this investigation is to evaluate the effect of mango and guava leaves on rats feed on high fat diets.

MATERIALS AND METHODS

Materials

Leaves of guava and mango were obtained, and identified by botanical members of the department of Botany, Faculty of Agriculture, Menoufia University

Methods

1- Proximate analysis

Moisture content, crude protein, ash, crude lipids were determined and total carbohydrates by **AOAC, (2000)**.

2- Determination of total phenolic compounds, total flavonoids and Quantitative identification of phenolic compounds .

A- Preparation of methanolic extract of guava and mango leaves:

Preparation of methanolic extract by (Mukhtar and Ghori, 2012).

B- Determination of total phenolic compounds:

The total phenolic compounds in each extract were determined colorimetrically by the method of Folin-Ciocalteu as described by (Gulcin *et al.*, 2002).

C- Determination of total flavonoids contents:

The total flavonoids contents were determined using the method reported by (Dewanto *et al*., 2002).

D- Quantitative determination of phenolic compounds in guava and mango leaves by HPLC

A modified method of (Zuo *et al*.,2002) was used .

3- Biological experiment

A- Experimental Design

Twenty five rats were divided into five groups: negative group fed on basal diet (46.7% starch, 23.3% sucrose, 12.5% casein, 10% corn oil, 3.5% salts mixture, 3% fibers and 1% vitamins mixture), groups (B, C, D and E) were allowed to feed hyperlipidemic diet (41.23% starch, 20.52% sucrose, 20% coconut oil, 12.5% casein, 3.5% salts mixture, 3% fibers, 1% vitamins mixture, 1% cholesterol and 0.25% cholic acid) to induce hyperlipidemic through the feeding period (Abozid *et al.,* 2015).

B- Blood sampling and analysis

ALT and AST activities were measured by Retiman and Frankel (1957). T.c was measured by Allain *et al.,* (1974); HDL-c was determined according to Lopez *et al.,* (1977); the T.G were analyzed by Fossati, and Prencipe (1982); LDL-c, risk ratio and atherogenic index were determined by Lopez *et al.,* (1977).

RESULTS AND DISCUSSION

Proximate analysis of guava and mango leaves

Data given in Table (1) show the chemical composition of guava and mango leaves. The obtained results indicated that: mango leaves contain moisture (65.15 %), ash (4.82 %), crude protein (18.45 %), crude fat (1.2 %) and total carbohydrate (75.53 %). Guava leaves contain of moisture (67.56 %), ash (5.47 %), crude protein (22.78 %), crude fat (1.09 %) and total carbohydrate (70.66 %). Theses result were in accordance with earlier findings reported by many authors; Alberts *et al.,* (2002) depicted that mango leaves consisted of (5.5%) ash and Fafiolu *et al.,* (2006) found that mango leaves consisted of protein (17.14 %).

Table (1): Proximate analysis of guava and mango leaves

Components	Percentage	
	Guava leaves	Mango leaves
Moisture	67.56	65.15
Ash	5.47	4.82
Crude protein	22.78	18.45
Crude fat	1.09	1.2
Total carbohydrate	70.66	75.53

Phenolic and flavonoids of guava and mango L.

Data presented in Table (2) showed that total phenolic compounds in guava leaves extract was 118.1 meanwhile, total flavonoids was 11, comparing with 73.7 and 9.75 of total phenolic compounds and total flavonoids, respectively in mango leaves extract. These results were in accordance with earlier findings reported by Donatus and Vitus (2008); Gazwi and Mahmoud (2019) and Elixabet and Antonio (2016).

Identification of phenolic compounds of guava and mango leaves by using HPLC

From Table (3), it was found that both guava leaves and mango leaves contains 14 phenolic compounds. Major compounds in guava leaves extract were gallic acid, catechin, chlorogenic acid, naringenin and ellagic acid; meanwhile catechin, pyro-catechol, gallic acid and methyl gallate were the major phenolic compounds in mango leaves. These results agree also with that of Laulloo *et al.,* (2018), who reported that the methanol extract of mango leaves are rich in gallic acid, ellagic acid and methyl gallate. Wang *et al.,* (2017), reported that, presence of gallic acid, rutin, chlorogenic acid, isoquercitrin and quercitrin in non-fermented guava leaves and fermented guava leaves.

1- *In vivo* study of the effect of guava and mango leaves on hyperlipidemic rats

A- The effect on lipids profile

The effect of adding guava and mango leaves to the diet of rats fed on hyperlipidemic diet compared with rats fed on normal diet for 30 days on the level of plasma lipid profile parameters (total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, risk ratio and atherogenic index) are illustrated in Tables (4 and 5). Data indicated that total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, risk ratio and atherogenic index in normal control group were 92.8, 128.2, 47, 20.16 mg/dl , 1.97 and 0.42 respectively, and in positive control group, total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, risk ratio and atherogenic index reached 253 , 297.6 and 32.2 , 161.28 mg/dl , 7.86 and 5.01 respectively. In treated groups (Groups C, D and E) After 30 days, reduced significantly both of plasma total cholesterol and plasma triglyceride to 155.8 and 101.3 mg/dl for group C, and reduced to 156 and 103.4 mg/dl for group D, and reduced to 127.2 and 97.4 mg/dl for group E, and increased significantly HDL-cholesterol level to 51.8, 44.6 and 50.8 mg/dl respectively, while LDL-cholesterol, risk ratio and atherogenic index reduced to 83.74 mg/dl, 3.01 and 1.62 for group C and reduced to 90.72 mg/dl, 3.49 and 2.03 for group D, and reduced to 56.92 mg/dl, 2.5 and 1.12 for group E. The higher reduction in total cholesterol levels in group treated with guava leaves (group D) may be due to the increased excretion of bile acid, guava leaves is reported to have high crude fiber (Yamashiro *et al.,* 2010).

The mango and guava leaves showed significant anti-hyperlipidemic activity which may be attributed due to presence of flavonoids and phenolics. The increase in HDL-cholesterol on administration of the plant samples is an indicator of the plant as a good antihyperlipidemic agent.

B- The effect on liver enzymes:

Data in Table (6) indicated that alanine transaminase (ALT), aspartic transaminase (AST) activities were 43.48 IU/L, 36.26 IU/L, respectively, for normal control group, and in positive control group, ALT and AST activities reached 92.72 IU/L, 221.66 IU/L, respectively. For treated groups (C, D and E) liver enzymes (ALT and AST) activities were 41.84 and 56.64 IU/L for group C, 32.18 and 38.44 IU/L for group D and 31.22 and 31.02 IU/L for group E. These results were according to Roy and Das (2011) and Jung *et al.,* (2013. Our results however run parallel with the finding of Patrick *et al.,* (2008) who reported the inhibitory effect of different plant extracts on the transaminases activity.

Table (2): phenolic and flavonoids of guava and mango L.

Active compounds	Guava leaves	Mango leaves
Total phenolic compounds (mg/g extract)	118.1	73.3
Total flavonoids (mg/g extract)	11	9.75

Table (3): quantitative of phenolic compounds of guava and mango leaves by using HPLC.

Phenolic compounds (µg/ml)	Guava leaves	Mango leaves
Gallic acid	770.12	775.99
Chlorogenic acid	410.26	ND
Catechin	473.97	1205.98
Methyl gallate	37.08	191.18
Coffeic acid	5.03	ND
Syringic acid	54.18	11.22
Pyro catechol	ND	925.34
Rutin	46.24	6.97
Ellagic acid	197.01	56.42
Coumaric acid	70.21	62.66
Vanillin	ND	25.97
Ferulic acid	43.45	17.42
Naringenin	300.49	86.20
Daidzein	0.91	43.68
Querectin	30.51	1.31
Cinnamic acid	0.92	1.82

ND: Not detectable.

Table (4): Effect of guava and mango leaves on T.C, T.G and HDL-c:

Groups	T.C (mg/100ml)	T.G (mg/100ml)	HDL-c (mg/100ml)
Group A	$92.8^{a} \pm 1.4$	$128.2^{a} \pm 2.6$	$47^{b} \pm 4.3$
Group B	$253^{b} \pm 5.2$	$297.6^{b} \pm 4.6$	$32.2^{a} \pm 3.2$
Group C	$155.8^{a} \pm 2.9$	$101.3^{a} \pm 3.1$	$51.8^{b} \pm 4.8$
Group D	$156^{a} \pm 2.7$	$103.4^{a} \pm 4.1$	$44.6^{b} \pm 4.4$
Group E	$127.2^{a} \pm 4.4$	$97.4^{a} \pm 2.3$	$50.8^{b} \pm 5.1$

G. A (normal), G.B (Positive), G. C (group treated with 10% mango leaves), group D (group treated with 10% guava leaves) and group E (group treated with 5% mango leaves +5% guava leaves).

Table (5): Effect of guava and mango leaves on LDL-cholesterol, risk ratio and atherogenic index.

Groups	LDL-cholesterol (mg/dl)	Risk ratio	Atherogenic index
Group A	$20.16^{a} \pm 3.8$	1.97	0.42
Group B	$161.28^{c} \pm 4.7$	7.86	5.01
Group C	$83.74^{b} \pm 3.2$	3.01	1.62
Group D	$90.72^{b} \pm 2.1$	3.49	2.03
Group E	$56.92^{b} \pm 3.4$	2.5	1.12

G. A (normal), G.B (Positive), G. C (group treated with 10% mango leaves), group D (group treated with 10% guava leaves) and group E (group treated with 5% mango leaves +5% guava leaves).

Table (6): Effect of guava and mango leaves on ALT and AST activities

Group	ALT activity (IU/L)	AST activity (IU/L)
Group A	$43.48^{a} \pm 2.8$	$36.26^{a} \pm 1.3$
Group B	$92.72^{b} \pm 2.3$	$221.66^{b} \pm 5.4$
Group C	$41.84^{a} \pm 2.3$	$56.64^{a} \pm 3.1$
Group D	$32.18^{a} \pm 1.2$	$38.44^{a} \pm 1.9$
Group E	$31.22^{a} \pm 2.1$	$31.02^{a} \pm 2.1$

G. A (normal), G.B (Positive), G. C (group treated with10% mango leaves), group D (group treated with10% guava leaves) and group E (group treated with 5% mango leaves +5% guava leaves).

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تأثير أوراق الجوافة والمانجو على الفئران المغذاة على نظام غذائي غني بالدهون

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الملخص العربي

صممت الدراسة الحالية لدراسة التركيب الكيميائي لكل من أوراق الجوافة والمانجو وتقييم التأثير المحتمل لها كعوامل خافضة للدهون في حيوانات التجارب. أظهر التحليل الكيميائي لأوراق الجوافة والمانجو احتوائهم علي: كربوهيدرات (٧٠,٦٦ و ٧٥,٥٣٪، علي التوالي)، بروتين خام (٢٢,٧٨ و ١٨,٤٥٪، علي التوالي)، رماد (٥,٤٧ و ٤,٨٢٪،علي التوالي) ودهون خام (١,٠٩ و ١,٢٪،علي التوالي). أما الفينولات الكلية في أوراق الجوافة والمانجو فكانت ١١٨,١و ٧٣,٧ مجم لكل جم، علي التوالي، أما الفلافونات الكلية فكانت ١١ و ٩,٧٥ مجم لكل جم، علي التوالي. أظهرت نتائج التحليل باستخدام HPLC احتواء كلا من أوراق الجوافة والمانجو علي ١٤ مركب فينولي، وكانت اهم المركبات في أوراق الجوافة حمض الجاليك والكاتشين وحمض الكلوروجينيك، أما في أوراق المانجو فكانت مركبات الكاتشين والبيروكاتيكول وحمض الجاليك هي أهم المركبات. تم إستخدام ٢٥ فأر لمدة ٣٠ يوم وقسمت الفئران إلى خمس مجموعات على أن تكون المجموعة الأولى كنترول سالب يتم تغذيتها على وجبة أساسية فى حين أن المجموعة الثانية يتم تغذيتها على وجبة عالية الدهون لتمثل المجموعة الكنترول الموجب والثلاث مجموعات الاخرى يتم تغذيتهم على وجبة عالية الدهون مصحوبة بإضافات من كلا من أوراق الجوافة والمانجو حيث عوملت المجموعة الثالثة بنسبة ١٠٪ أوراق مانجو والمجموعة الرابعة عوملت بنسبة ١٠٪ أوراق جوافة والمجموعة الخامسة عوملت بنسبة ٥٪ اوراق مانجو + ٥٪ أوراق جوافة . بنهاية التجربة تم تجميع عينات الدم لتقدير مستوى الليبيدات بالدم وتقدير إنزيمات الكبد. من النتائج المتحصل عليها يمكن الإشارة إلى أن مجموعة الكنترول موجب كانت الأكثر في مستويات الدهون الضارة بالدم (الجلسريدات الثلاثية – الكلوسترول الكلي – الكولسترول في الليبوبروتينات المنخفضة الكثافة)، كما لوحظ أن إستخدام أوراق الجوافة والمانجو أدى إلى نقص معنوي في مستويات الجلسريدات الثلاثية – الكلوسترول الكلي – الكولسترول في الليبوبروتينات المنخفضة الكثافة مما يشير إلى أن إستخدام كلا من الجوافة والمانجو مفيد فى علاج إرتفاع الدهون بالدم.