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Influence of yerba mate tea (Ilex paraguariensis) in improving some lipolytic enzymes of high-fat diet-induced obese rats

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Abstract

The present study aimed to evaluate tea beverage prepared from dried yerba mate leaves (*Ilex paraguariensis*) for its acceptability, identify and quantify its polyphenolic compounds, and its effects on obese rats .Tea prepared from yerba mate dried leaves was acceptable. The ethanolic extract of mate tea showed that, Chlorogenic acid was the main phenolic compound followed by caffeine ,Caffeic acid and the most important flavonoids was rutin, kamferol and quercetin have been identified and quantified using HPLC technique . Thirty five mature male rats weighted (160±5g) were randomly divided into two main groups. Group (1) (n=7) rats was fed on basal diet and kept as negative control (-ve) while, rats of the second main group (28 rats) were fed on a high fat diet containing 45% fat for 4 weeks to induce obesity , then divided into four sub groups(7rats each) ,sub group (1) was left as positive control (+ve) and subgroups (2), (3) and (4) were orally given yerba mate extract (1. 2 and 3 ml/100gb.wt) , twice daily for 8 weeks respectively. The rats were weighed; weight gains and feed efficiency ratios were calculated. At the end of experiment, all rats were sacrificed and adipose index was calculated. Blood samples were collected for biochemical analysis of lipid profile , phospholipids, hepatorenal function , and estimation of serum levels of glucose ,insulin, leptin and malondialdehyde (MDA) ,reduced glutathione (GSH) as well as the activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase(CAT) and pancreatic lipase were measured as well as histopathology of liver was done .The results showed that daily administration of yerba mate tea to obese rats significantly decreased weight gain (BWG%),daily food intake(FI) and (FER),adipose index, serum levels of TC ,TG LDL-c, VLDL-c, phospholipids, glucose, leptin level , AST ,ALT, ALP, blood urea nitrogen, uric acid concentration,(MDA) and caused an inhibition to pancreatic lipase while an increase in the level of HDL-c ,insulin level , reduced glutathione (GSH) content and the activity of the antioxidant enzymes (SOD, GPX, CAT) compared with that of the positive control . As well, it alleviated the histopathological changes which seen in liver of obese rats. In conclusion , intake of yerba mate tea may benefit patients who suffer from hyperlipidemia .

Introduction

Obesity is an excessive fat accumulation in the body that results from an imbalance between energy intake and energy expenditure associated with genetic, metabolic, and behavioral components. Despite of a major contribution of genetic susceptibility, the rapid development of obesity might reflect substantial changes of other factors such as dietary habit ; ***(Power and Schulkin 2008)***.Obesity leads to increase production of several inflammatory cytokines, which play a critical role in obesity-related inflammation and metabolic pathologies such as cardiovascular diseases, hypertension and dyslipidemia and diabetes mellitus;***(Jiao et al., 2009).*** The effects of obesity treatment can be maximized when diet control is accompanied by exercise and lifestyle changes. Furthermore, a variety of functional food and dietary supplements are available to help those who want to lose weight***(Pillitteri et al., 2008).***

One of the most important strategies in the treatment of obesity includes the development of nutrient digestion and absorption inhibitors, in an attempt to reduce the energy intake through gastrointestinal mechanisms, without altering any central mechanisms ***;(Shi and Burn 2004).*** Pancreatic lipase (PL) inhibition is one of the most widely studied mechanisms used to determine the potential efficacy of natural products as anti obesity agent ; ***(Birari and Bhutani 2007).*** Clinical approved drugs for obesity treatment, have been shown to act by inhibiting PL have certain unpleasant gastrointestinal side effects such as oily stools, oily spotting, and flatulence, among others, so the natural products for the treatment of obesity is an excellent alternative strategy for the development of safe and effective anti obesity drugs; ***(Bhutani et al., 2007).*** Several Dietary bioactive components including those that are derived from plant sources such as polyphenols and certain fatty acids, are reported to suppress both systemic and adipose tissue inflammation and potentially improve these obesity-associated metabolic disorders ***(Afolayan and Mbaebie 2010).***

Yerba Mate tea (*Ilex paraguariensis*) is an herbal tea beverage made from the leaves of the tree *Ilex paraguariensis* St. Hil var. *paraguariensis* (Aquifoliaceae). It is consumed widely in South America, including Argentina, Brazil and Paraguay. The indigenous people have used it for centuries as a social and medicinal beverage and an ingredient in dietary supplement industries . It is gaining rapid penetration into world markets, including the United States and China, commercially packed in individual tea bags; ***(Kang et al., 2012).*** Yerba mate is also considered a functional food, because of its nutritional and medicinal properties, such as hypocholesterolemic, hepatoprotective, diuretic, and antioxidant effect; which can protects against the harmful effect of free radicals and increases the defense system of the organism ***(Lorena et al ., 2013) and (Eloir et al .,2015)*** .These health benefits have been attributed to phenolic compounds, which are major constituents of *(Ilx paraguariensis)* ***,(Samuel et al., 2013)*** .

The main Polyphenols present in mate are Caffeoyl derivatives (Chlorogenic acid , 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid) and Caffeic acid moreover caffeine and Theobromine, and a minor content of flavonoids (Quercetin, Kaempferol, and Rutin). ***(Filip et al ., 2009)*** . Polyphenolic compounds found in mate tea differ significantly from green tea because mate tea contains high concentration of Chlorogenic acid and no catechins; in addition to Polyphenols Yerba mate leaves contain about 5 to 10 % of its dry weight Saponin which is phytochemicals that have been found to specifically stimulate the immune system and aid the body in protecting against disease . ***(Carlos et al., 2015)***

(Puangpraphant et al ., 2013) concluded that a high content of mineral elements, especially K, Mg, and Mn, in mate is considered a great relevance" to the nutritional value of Mate infusions . ***Harrold et al., (2013) and (Pimentel et al., 2013)*** reported that mate extract may have beneficial effects in the management of obesity by decreasing food intake , delayed gastric emptying, suppressing appetite ,decreased the perceived time to fullness and ultimately induced a significant weight loss after 45 d. Furthermore, the affects of yerba mate on lipid metabolism included reductions of serum cholesterol, serum triglycerides, and glucose concentrations in mice that were fed a high fat diet. Its effects on cholesterol levels could be partially attributed to its Saponin content and potentially can be used to treat obesity and diabetes. ***Lima et al.,(2014) and Alessandra and Marcelo. (2015)***

The present study aimed to evaluate tea drink prepared from dried yerba mate leaves (*Ilex paraguariensis*) for its acceptability, indentify and quantify its phenolic compounds and to study the effect of oral administration of yerba mate tea (MT) on body weight loss, body fat reduction and several enzymes and hormones connected to overweight of high-fat diet-fed rats.

Materials and Methods

Plant

The dried yerba mate leaves (*Ilex paraguariensis*) were purchased as crude dried material from a local company for folk Medicinal Plants and Herbs, Cairo , Egypt .

Preparation of yerba mate tea

The dried leaves were milled using a coffee grinder into a fine powder .Yerba mate tea was prepared by using 10g fine powder /100ml distilled water and boiling for 5 min at100 °C The solution was kept to stand for 10min before being filtered , cooled to room temperature and adjusted to 100ml water before using ***(Renno et al., 2006)*** .Rats received tea at level of 1,2 and 3ml/100g b .wt).

Sensory evaluation

Tea was prepared freshly in boiled water at the concentration 10g/100 ml and kept in thermo bottles, and was served warm during the different tests . Then sensory acceptance test expressed as taste ,color, aroma, appearance and overall acceptability was evaluated by ten randomized volunteers***(Ekissi et al., 2014)***

Determination of phenolic compounds:

The ethanolic extract of poly phenolic compounds were fractionated ,identified and determined by HPLC according to ***(Goupy et al., 1999).***

Preparation of basal diet:

Basal diet was prepared according to the method of ***Reeves et al., (1993).*** It was consisted of 20 % protein (casein), 10 % carbohydrate, 4.7% fat (corn oil), 0.2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch up to 100 %.

Animals.

Thirty five mature male albino rats of Sprague Dawley strain weighing (160±5g) and 10–12 weeks old were purchased from Laboratory of Animal Colony Helwan Egypt. Rats were maintained under controlled hygienic conditions. . Animals were housed in clean cages, kept under controlled hygienic conditions and maintained at room temperature at 25 ± 2 °C, relative humidity of 50 ± 5% and photoperiod of 12 hr dark/12 hr light cycles .Animals were fed on basal diet and water was provided *ad libitum*. Rats were allowed to acclimatize to the laboratory environment for 7 days before starting of the experiment.

Induction of obesity

Obesity and acute hyperlipidemia was induced by feeding rats on high fat-diet (HFD) which supplies 45 % calories from fat (sheep bile fat) for 6 weeks according to ***Bhatt et al.,(2006),*** while normal basal diet supplies 11% calories from fat (corn oil).

Experimental design

After one week adaptation period, the rats were randomized into two main groups. Group (1) (n=7) rats was fed on basal diet and kept as negative control (-ve) group while, rats of the second main group (28 rats) were fed on a high fat diet containing 45% fat for 4 weeks to induce obesity , then divided into four sub groups ,sub group (1) was left as positive control (+ve) group fed only on basal diet and subgroups (2), (3) and (4) were orally given yerba mate extract (1. 2 and 3 ml/100gb.wt) , twice daily for 8 weeks respectively and fed on basal diet . Feed intake was calculated daily and body weight gain was recorded weekly. Feed efficiency ratio was calculated according to ***Chapman et al., (1959)*** as FER = weight gain (g) / feed intake (g). At the end of the experiment, the rats were anesthetized by prolonged

exposure to ether . Blood samples were withdrawn by cardiac puncture into clean centrifuge tubes. Blood was left standing for 10 minutes to clot and then centrifuged at 4000 rpm for 15 minutes for separating the serum which was kept frozen till biochemical analyses. Left and right inguinal adipose pads were removed and weighed. The sum of adipose pads to body weight, multiplied by 100, yielded adiposity index ***Jeyakumar et al .,(2006).*** In addition, livers of the sacrificed rats were removed for histopathological study.

Biochemical analyses

Serum total cholesterol ***Ratliff and Hall, (1973),*** triglycerides ***Jacob and Van-Denmark, (1963)*** and high density lipoprotein ***Richmond, (1973)*** were chemically measured. Low density lipoprotein (LDL) was calculated ***Friedewald et al., (1972).***). Leptin was measured using enzyme –linked immunosorbent assay (ELISA) according to ***Xiong et al., (2005).*** Lipase enzyme activity was measured as mentioned by ***Lott (1986).*** Serum phospholipids were determined according to the methods of ***Holman (1943).*** Serum glucose levels were determined according to the methods of ***Trinder (1969).***Insulin level was measured according to ***Tempel et al., (1992).*** Activities of serum liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) were chemically determined according to ***Bergmeyer et al. (1978)*** and alkaline phosphatase (ALP) according to ***Roy (1970)*** Blood urea nitrogen was determined using BioMérieux kits according to ***Patton and Crouch (1977).*** Serum uric acid ***Fossati et al., (1980)*** and creatinine concentrations were chemically determined ***Husdan and Rapoport, (1968).***

Serum MDA level as µmoles/dL. was determined as described by ***Draper and Hadley (1990).*** Serum reduced glutathione concentration (GSH) as µmoles/dL. was measured by the method described by ***Beutler et al.,(1963)*** Serum activity of SOD,GPX and CAT enzymes were determined according to the methods described by ***Kakkar et al.,(1984) and Sinha et al .,(1972):***

Histopathological examination:-

Liver of the treated rats were taken and fixed in 10 %(v/v) neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were then cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H&E) then examined microscopically according to ***Carleton ,(1979).***

Statistical analysis

Data were presented as means ± SD. Statistical analysis was performed with one way analysis of variance (ANOVA) test followed by Duncan's multiple range test ***(Snedecor and Cochran, 1986)*** using computerized Statistical Package of Social Sciences (SPSS) program.

Results

Preliminary study of yerba mate tea at concentration 5 and 10 %

Data presented in table (1) show that dried yerba mate tea prepared using different concentration (5 or 10%) of as preliminary study to evaluate its sensory characteristics. A significant difference in taste, color and overall acceptance were found between the two concentration ,while there was no significant different in aroma .Tea prepared using 10% concentration was more acceptable to all ten volunteers, so this concentration was used in the experiment of biology.

Table (1):
Sensory evaluation of of yerba tea (MT)

Groups	Taste	Color	Aroma	OA
MT at 5%	8.24±0.55b	9.96±0.27b	8.74±044a	8.00±0.25b
MT at 10%	9.22±0.22a	7.06±01.7a	9.08±0.44a	9.13±0.09a

Data in table (2) shows the concentration in milligrams/gram of polyphenolic compounds of yerba mate tea at 10% as follows Chlorogenic acid (20.9), Caffeine (7.82), Caffeic acid (5.82) Theobromine (3.30) and Chlorogenic acid related compounds (84.82) were the most abundant Phenolic compound. Also there were three fractionated flavonoids compound namely Rutin (14.7), Quercetin (3.3) and Kamferol (1.2) in milligrams/gram .

Table (2):
Identification and determination of phenolic compounds in yerba mate tea determined by HPLC.

Phenols compound	Concentration mg/g	Flavonoid compound	Concentration mg /g
Chlorogenic acid	20.91	Rutin	14.7
Caffeic acid	5.82	Quercetin	3.3
Caffeine	7.82	Kamferol	1.2
Theobromine	3.30		
Chlorogenic related compound	84.82		

Table(3): showed that feeding rats on high fat-diet (HFD) that supplied 45% calories from fat (sheep's bile fat) for 8 weeks caused significant increases ($P< 0.05$) in body weight gain, feed intake , feed efficiency ratio (FER) , adiposity index and leptin when compared to the control - ve group.

Oral administration of yerba mate tea (MT) at (1 , 2 and 3 ml/100g b. wt) caused significant decreases ($P<0.05$) in body weight gain, feed intake , FER , adiposity index as compared to the positive control group fed on(HFD) . Yerba mate tea at (3ml/100g b. wt) caused The highest reduction of FI, BWG, and adiposity index by about 24.87% 59.50 % and 60.76% respectively when compared with the control positive group (+ve) .

Table (3):

The effect oral administration of yerba mate tea on feed intake (FI) body weight gain efficiency ratio (FER), and Adiposity index of obese rats.

Groups	FI g/day)(	BWG g/day)(	FER	Adiposity index
Negative control	19.10± 1.36a	2.27± 1.34 a	0.118± 0.89b	0.511± 0.032c
Positive control	28 .42± 1.31c	5.26± 2.11 c	0.145± 1.6a	1.56± 0.071a
MT at (1ml/100g b.wt)	22.34± 1.31b	2.56± 1.11a	0.114± 0.84a	1.13± 0.031a
MT at (2 ml/100g b.wt)	22.13± 1.32b	2.46± 1.12a	0.111± 0.84b	0.891± 0.42b
MT at (3ml/100g b.wt)	21.35± 1.43b	2.13± 1.31a	0.0997± 0.91c	0.612± 0.041c

Mean ± SD values in each raw with different superscripts (a, b, c,) are significantly different as compared to the control groups at *P*<0.05 n = 7 rats /group .

Data presented in table (4) showed that feeding high fat diet resulted in significant increases (*P*< 0. 05) in serum levels of triglycerides (TG) , total cholesterol (TC), low density lipoprotein (LDL) and very low density lipoprotein (VLDL), and an increase in Phospholipids and lipase enzyme when compared to the negative control group. Oral administration of yerba mate tea at 1 , 2 and 3 ml/100 g b. wt resulted in significant decreases(*P*< 0. 05) in the elevated serum TC , TG , LDL and VLDL levels and significant increase in HDL –c.

Oral administration of yerba mate tea at (3ml 100g b wt) caused the highest decrease by 30.09 % ,49.21 , 70 .3 and 49.2 %to TC,TG, LDL and VLDL respectively while the same dose of yerba tea caused the highest increase in serum HDL by 43.7% when compared to the positive control (+ve) group .

The obtained results of lipase and phospholipids showed a value of 85.61 and 125,0 respectively due to feeding high fat diet (+vel) as shown in the same table which was 1.5 -2 times as that of (-ve). Administration of yerba mate tea resulted in gradual and significant decrease in pancreatic lipase by about 12.3%, 31.1% and 52%resppectively and phospholipids by 5.3 %,8.87%and 15.99%respectively

Table (4) :

Effect of oral administration of yerba mate tea on lipid profile ,lipase and phospholipids of obese rats.

Groups	TC (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)	Lipase (mg/dL)	Phosholipid (mg/dL)
Negative control	93.471± 1.87a	54.33± 1.41c	66.243± 2.44a	16.361± 1.89d	10.866± .32c	48.31± 2.45c	85.00± 50.2d
Positive control	135.959± 1.09d	118.365± 2.05a	43.562± 1.98d	68.724± 2.22a	23.673± .22a	85.61± 6.6a	125.00± 41.1a
MT at (1ml/100g b.wt)	125.082± 2.13c	100.64± 1.11ab	49.522± 1.23d	55.432± 2.11b	20.128± .36a	75.05± 1.34b	118.31± 33.2b
MT at (2 ml/100g b.wt)	115.356± 1.17c	88.91± 3.51ab	55.624± 1.59c	41.946± 1.98c	17.782± .64b	45.91± 1.44 c	113.91± 64.3c
MT at (3ml/100g b.wt)	95.038± 2.19b	58.105± 1.23b	62.634± 1.37a	20.383± .87cd	12.021± .23c	35.24± 3.91d	105.01± 11.1c

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significantly different as compared to the control groups at *P*<0.05 n = 7 rats/group

From data in Table (5) rats fed on high fat diet had significant increases (*P*< 0. 05) in serum glucose level and leptin hormone when compared to the negative control group. Oral administration of yerba mate tea at concentration (1, 2 and 3 ml/100g) for 8 weeks resulted in significant decrease (*P*< 0. 05) in elevated serum glucose level due to the increases of insulin secretion by 8.07% ,25.4% and 43.76% respectively and reduction of leptin hormone by about 33.43%, 36.99% and 40.49% respectively

Table (5):

The effect of oral administration of yerba mate extract on glucose ,insulin and leptin hormone of obese rats

Groups	Glucose mg/dl	Insulin ng/d	Leptin (ng/ml)
Negative control	92.00±3.65d	67.56±0.36 a	30.81±0.32 a
Positive control	152.02±3.1a	46.43±1.91 c	53.68±1.35d
MT at (1ml/100g b.wt)	120.13±3.03b	50.18±1.16 c	35.84±2.16 b
MT at (2 ml/100g b.wt)	112.03±1.53c	58.25±2.16 b	33.82±0.32 b
MT at (3ml/100g b.wt)	95..53±1.24d	66.75±2.97 a	31.94±3.21 a

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significantly different as compared to the control groups at *P*<0.05 n = 7 rats

Data recorded in Table (6): showed that rats fed on high fat diet had significant increases ($P< 0.05$) in serum liver enzyme AST, ALT and ALP , when compared to the negative control group.

Oral administration of yerba mate tea at (1, 2 and 3 ml/100g b. wt) for 8 weeks resulted in significant decreases ($P< 0.05$) in the elevated serum AST, ALT and ALP when compared to the positive control (+v) group. High dose (3m/100g b wt) of Oral administration of yerba mate tea caused the highest decrease in the elevated serum liver enzymes compared to control positive group by 33.38% for AST, 36,09% for ALT and 36. 57% for Alp respectively.

Table (6):

The effect of oral administration of yerba mate extract on serum liver enzymes (AST) , (ALT) and (ALP) in obese rats

Groups	AST (U/L)	ALT(U/L)	ALP(U/L)
Negative control	27.285± 2.231e	.24.016± 2.07e	49.41± 1.612 e
Positive control	45.412± 1.66a	50.543± 2.62a	81.24 ± 2.45 a
MT at (1ml/100g b.wt)	41.601± 2.42b	43.353± 1.69b	68.23 ± 039b
MT at (2 ml/100g b.wt)	36.648± 1.98c	37.502± 1.34c	75.12 ± 040 c
MT at (3ml/100g b.wt)	30.252±1.78d	32.297± 1.56d	51.64 ± 1.55d

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significantly different as compared to the control groups at $P<0.05$ n = 7 rats.

Results presented in table (7) revealed that rats fed on high fat diet had significant ($P<0.05$) increases in levels of blood urea nitrogen (BUN), uric acid (UA) and creatinine (Cr.) when compared to the negative control group. Oral administration of yerba mate tea at concentration at (1, 2 and 3 ml/100g b.wt) to obese rats led to significant ($P< 0.05$) decreases of the above mentioned parameters as compared to the positive control (+ve) group .

Table (7):

The effect of oral administration of yerba mate tea on serum urea nitrogen, uric acid and creatinine concentration in obese rat

Groups	BUN(mg/dL)	UA(mg/dL)	CR(mg/dL)
Negative control	23.245± .745c	2.02 ±1.782d	.57 ±.043a
Positive control	39.43± 1 .62a	3.422 ±1.76a	1.34± .076b
MT at (1ml/100g b.wt)	34.562±1 .98bc	2.992 ±.83b	.942 ±.018b
MT at (2 ml/100g b.wt)	29.452± 2.21b	2.434± .74bc	.873± 1.13bc
MT at (3ml/100g b.wt)	24.224± 1.81c	2.22 ±1.45d	.61±0.89 d

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significantly different as compared to the control groups at $P<0.05$ n = 7 rats/group

Results illustrated in table (8): showed that rats fed on high fat diet (+ve) had decreased of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) enzymes activities in serum as compared with the negative control group. Oral administration of high dose of yerba mate tea to obese rats for 8 weeks caused gradual significant increase in the antioxidant enzyme activities in serum by 55.5% , 79.16 and 131.9 respectively for (GPX) , 7.2% , 30.29 % and 41.75% for SOD and 12.9%,31.64 and 47.58% for (CAT) as compared to the control positive group.

Table (8) :

Effect of oral administration of yerba mate tea on the activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in serum in obese rats .

Groups	GPx (n moles)	SOD (U/mg protein)	CAT (n moles)
Negative control	19.1±0.71 a	93.56±0.36 a	65.81±0.32 a
Positive control	7.2±0.65d	65.43±1.91 d	44.68±1.35d
MT at (1ml/100g b.wt)	9.2±0.67 c	70.18±1.16 c	50. 45±2.16 c
MT at (2 ml/100g b.wt)	12.9±0.91 b	85.25±2.16 b	58.82±0.32 b
MT at (3ml/100g b.wt)	16.7±0.72 a	92.75±2.97 a	65.94±3.21 a

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significantly different as compared to the control groups at $P<0.05$ n =7 rats/group

Results in table (9) showed that rats fed on high fat diet had a significant increase in lipid peroxide malondialdehyde (MDA) content and decrease in level of reduced glutathione (GSH) when compared with the negative control group Oral administration of yerba mate tea at 1 , 2 and 3 ml /100gb .wt significantly decreased MDA content and increased GSH content in the serum when compared with the positive control group . The highest decrease of (MDA) was to the group orally given 3ml /100g .b wt by 49. 16 %and the highest increase of (GSH) was to the same group by 47.41%. from above results It could be noticed that ,there was a negative relation between MDA and GSH . where MDA decreased and GSH increased due to administration of yerba mate tea.

Table (9) :

Effect of oral administration of yerba mate tea on malondialdehyde (MDA) and reduced glutathione (GSH) contents in serum of obese rats.

Groups	MDA (µmol/dl)	GSH (µmol/dl)
Negative control	1.25±0.02e	41.27±0.02a
Positive control	2.99±0.01a	27.44±0.01e
MT at (1ml/100g b.wt)	2.02±0.01b	32.36±0.17d
MT at (2 ml/100g b.wt)	1.92±0.01c	37.25±0.10c
MT at (3ml/100g b.wt)	1.52±0.01d	40.54±0.01b

Mean ± SD values in each raw with different superscripts (a, b, c, d) are significantly different as compared to the control groups at $P<0.05$ n = 7 rats/group

Histopathological study

Examination of liver of normal rats fed on basal diet showed normal histological structure of hepatic lobules (photo1). Liver of obese rats fed on a high diet revealed marked congestion of hepatic central vein (photo 2) Oral administration of yerba mate tea at 1and 2 ml/100g.bwt for 8 weeks to obese rats to obese rats showed moderate congestion of central vein and hepatic sinusoids(photo 3, 4).in obese rats given 3ml/100g b. wt of yerba mate tea ,the examination of liver sections showed only mild congestion of central vein (photo 5).

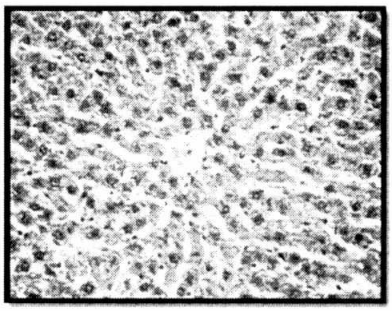


Photo (1)
liver of(- ve) control rat showing normal histological structure of hepatic loubles

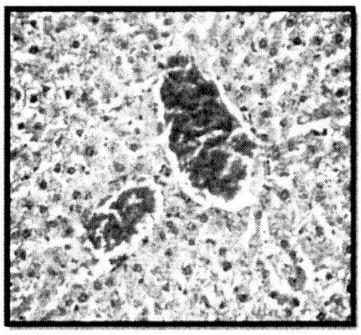
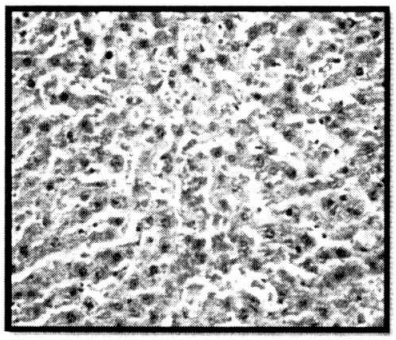
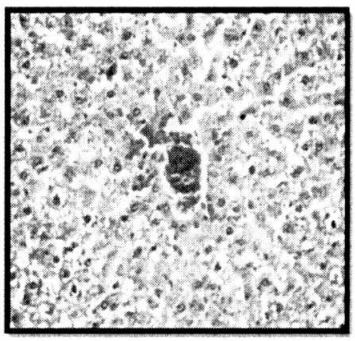


Photo (2)
liver of an obese (non treated) rat showing marked congestion of hepatic central veins



Photo(3)

liver of an obese rat given1ml/100 g b.wt for 8 week showing moderate congestion Of central vein) and hepatic sinusoids



Photo(4)
liver of an obese rat given Yerba ate tea at 2m/100g b .wt for 8week showing moderate congestion Of central vein) and hepatic
sinusoid

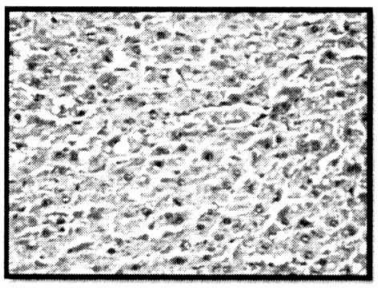


Photo (5)
liver of an obese rat given Yerba mate tea at 3m/100g b .wt for 8week showing only mild congestion Of central vein

Discussion

The goals of this study were to evaluate the effect of oral administration of yerba mate tea MT (*Ilex paraguariensis*), on weight loss of obese rats fed on high-fat diet. Tea prepared from dried yerba mate leaves was more acceptable by all ten volunteer . There are three Xanthines found in yerba mate, caffeine, Theobromine, and Theophylline, which give yerba mate its bitter, flavor characteristic and stimulant effects ***Athayde et al., (2007);*** Yerba mate leaves have a relatively high saponin content, 5 to 10% of the total dry weight. ***Puangpraphant et al., (2013).*** One common negative factor associated with the use of mate extracts as antimicrobial food preservatives is their effect on food sensory (flavor, odor) properties. The flavor of yerba mate infusions has been described in various terms, such as bitter, acid, astringent, hay, green, humid and toasted ,However, use of yerba mate (dried and aqueous extracts) had no effect on the taste or smell of precooked chicken meat balls.

The HPLC fractionation and determination of poly phenolic compounds of yerba mate tea in the present study displayed that Chlorogenic acid , Caffeine , Caffeic acid , Theobromine ,Chlorogenic acid related compound ,Rutin, Quercetin and Kamferol were the most abundant phenolic compounds. These data were to some extent similar to those reported by ***Laura et al.,(2007) ,Kellie et al ., (2012) Lorena et al., (2013), Carlos et al.,(2015)*** .The previous authors mentioned that Yerba mate is rich in several bioactive compounds such as Polyphenols (Chlorogenic acid), Xanthines (caffeine and Theobromine), purine alkaloids (Caffeic acid, 3,4-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid), flavonoids (Quercetin, Kaempferol, and Rutin), and saponins, which are absorbed by the body and may act as antioxidants or as free radical scavengers. The caffeoylquinic acids or dicaffeoylquinic acids, generally known as Chlorogenic acids (CGAs) which seem to have antitumor activity as well as the ability to inhibit carcinogenesis, are the main Polyphenols in yerba mate .The level of polyphenolic in yerba mate extracts are greater than those of green tea and similar to levels found in red wine ***(Gugliucci et al., 2009)*** .Yerba mate extracts are highly rich in Chlorogenic acids, and unlike green tea, contain no catechins ***Chandra and De Mejia., (2004).***

Mate has central nervous system-stimulant properties that are attributed to its methylxantines alkaloids, such as caffeine and Theobromine***(Athayda et al., 2007)*** and it is known to contain compounds with antioxidant properties, such as phenolic acids and caffeoylquinic acid derivatives, which are the most abundant compounds in the leaves ***Heck and de Mejia, (2007) and Bastos et al., (2007b)*** Other reported effects, including hepatoprotective, choleretic, diuretic, hypocholesterolemic, anti rheumatic, antithrombotic, anti-inflammatory, anti-obesity, and cardio protective effects, may partially be explained its popularity ***Bracesco et al., (2011).*** Yerba Mate may have benefits over other weight-loss herbal medicines and supplements, the use of which has been clinically linked to adverse events***(Pittler., 2005) .***

The result of this study showed that oral administration of mate tea caused a significant reduction of food intake, weight gain, adiposity index and decreased leptin level in rats fed on HFD compared to control negative group . These results agree with ***Kellie et al.,(2012) and Ruthl et al., (2009)*** who reported that the decrease in energy intake (food intake) , increase energy expenditure during the supplementation with yerba mate refer to the increase in the activation of the sympathetic nervous system (SNS),caused by its Caffeine content (a conditional appetite suppressor) According to **Andersen *and Fog (2001),*** in overweight patients, yerba mate extract significantly delayed gastric emptying, decreased the perceived time to fullness and ultimately induced a significant weight loss after 45 d.

The metabolic effects of mate extract appear to include the ability to maintain aerobic breakdown of carbohydrates during exercise for long periods of time. As a result, more calories are burned, thereby increasing cardiac efficiency and delaying the build-up of lactic acid ***Bracesco et al.,(2011)***

In the present study, the level of leptin hormone in the serum was elevated in the control positive group due to a high-fat diet. The treatment with Yerba Mate tea recovered the concentration of leptin to near the normal level . There is a relation between the level of leptin and obesity . ***Yudkin et al.,1999)*** illustrated that, obesity is associated with chronic mild inflammation that plays an important role in metabolism and homeostasis by secreting several hormones and signaling substances such as leptin with different protein structures and a number of biological functions as; satiety and appetite control, glucose and lipid metabolism, blood pressure regulation and inflammation and immune modulation .

Leptin, is an important hypothalamic satiety signal has been positively associated with the amount of body fat, a reduction in body weight could lead to a reduction in leptin levels, as reported by ***Ioffreda et al., (1998) and Bastos et al.,(2007a).***

Oral administration of yerba mate tea caused significant decrease in blood glucose level due to increasing the secretion of insulin hormone and decreasing adipose tissue. These results are in correlated with ***Kang et al., (2012)*** who showed that the ability of yerba mate to decrease the differentiation of pre adipocytes and reduce accumulation of lipids in adipocytes, both of which contribute to lessened growth of adipose tissue, lower body weight gain, and decreased obesity. ***Arcari et al. (2011)*** also demonstrated that treatment with yerba mate extract has potent anti-obesity effects in adipose tissue in vivo by controlling the expression of several genes related to obesity processes.

In this study, the oral administration of yerba mate tea caused a significant inhibition of the pancreatic lipase (PL) . The principal lipolytic enzyme synthesized and secreted by the pancreas, plays a key role in the efficient digestion of triglycerides. The phenolic compound in yerba mate tea has important role in this inhibition as explained by ***Mukherjee ., (2003) and Power and Schulkin (2008).***

PL is responsible for the hydrolysis of 50–70% of the total dietary fats. It removes fatty acids from the α and α′ positions of dietary triglycerides, yielding β-monoglycerides and long chain saturated and polyunsaturated fatty acids as the lipolytic product. Many polyphenolic such as flavones, tannins, and Saponin are active compound against PL activity ***Bixby et al., (2005) . Nakai et al., (2005)*** concluded that , Saponins delay intestinal absorption of dietary fat by inhibiting pancreatic lipase activity, which shows their hypolipidemic effects and Caffeine was found to enhance nor adrenaline-induced lipolysis in adipose tissue .Caffeoylquinic acids inhibit maltase and prolong the absorption of caffeine .

Hyperlipidemia is a major risk factor associated with atherosclerosis and coronary heart disease. Reducing this factor has been shown to be beneficial in humans for preventing stroke, cardiovascular diseases, and acute cardiac events. In this study the oral administration of yerba tea has a role in lowering total cholesterol, triglyceride, reducing the risk of cardiovascular disease. These results a greed with the results of ***Jisook et al.,(2008) and Borges et al .,(2013)*** which showed that rats fed on hypercholestrolemic diet for 30 days and at the last of 15 days were treated with *Ilex paraguariensis* extract (300mg/kg) caused a reduction on serum Cholesterol by 30% and triglyceride by 60.4% levels as compared to those fed on hypocholesterolemic diet alone. Similar results were recently reported by. ***Haslam and James (2005)*** who suggested that the *Ilex Paraguariensis* extract might have a protective effect against HFD-induced obesity in rats through an enhanced expression of uncoupling proteins and elevated AMPK phosphorylation in the visceral adipose tissue .

Saponin, an important compound found in *mate*, has been reported to interfere with cholesterol metabolism and provide a hypocholesteremic effect by inhibiting the passive diffusion of colic acid through the formation of micelles preventing absorption, anticancer, ant parasitic ***Taketa et al., (2004).***

Chlorogenic acid, the main Polyphenols in *yerba maté*, is thought to modulate the activity of glucose-6-phosphatase,which is involved in glucose metabolism ***klein et al .,(2011)*** and reduce the risk of cardiovascular disease

by decreasing oxidation of LDL and cholesterol, ***Edson et al.,(2008).*** In addition to Chlorogenic acid, the presence of methylxantines is also thought to account for some of the pharmacological effects of yerba mate ***(Mosimann and filho 2006).***

The data presented in this study suggested that the compounds found in yerba mate extract may act synergistically to suppress food intake , body weight gain, and decrease serum levels of cholesterol, triglycerides, LDL cholesterol, VLDL –C and glucose level and increase the HDL-C as well as increase the insulin level . These factors are the major players in metabolic syndrome and associated disorders .These results are in agreement with **Bastos *et al* .,*(2007a) and(Terezinha and Abdalla 2006)*** who concluded that Yerba mate has been reported to have various biological activities which have been mainly attributed to its high polyphenolic content. .

Yerba maté has been shown to inhibit the formation of advanced glycation end products (AGEs), with an effect comparable to that of two pharmaceutical grade AGE inhibitor drugs. ***Lunceford and Gugliucci (2005)*** reported that Polyphenols rich *paraguariensis* extracts are capable of inhibiting AGEs (or Millard reaction products) on a protein model in vitro whereas green tea displays no significant effect. Glycation, the non enzymatic adduct formation between sugar aldehydes and proteins, is one key molecular basis of diabetic complications due to hyperglycemia. The AGEs, which are irreversibly formed, accumulate with aging, atherosclerosis, and diabetes mellitus ***Andrade-Cetto and Wiedenfeld (2001).*** Our study showed results similar to that of the previous studies.

Oral administration of yerba mate tea (MT) to obese rats significantly reduced serum markers of liver function AST, ALT, ALP. and BUN, UA , CR. The serum aminotransferase reports the degree of injury to hepatocyte, since its release in the serum can mean cell death. Serum ALT is the best indicator for assessing the integrity of the liver cell . reducing the element of BUN , UA and CR, in the blood as elevated level of this element in blood indicates the presence of renal diseases that reduce the excretion of products, and show drug abuse and use of certain medications . These result were in accordance with ***Boaventura et al., (2012)*** who showed that *(Ilex paraguariensis)* is able to interfere in the circulatory system, acting as a diuretic and hypotensive agent. The chronic ingestion of aqueous extract of (*Ilex Paraguariensis*) promoted a decrease of ATP, ADP and pathway .modulating the balance in the purine levels which can induce relevant effects.

An important result in this study is , the reduction of lipid peroxidation Levels of malondialdhide (MDA), in the groups treated with (MT) , (MDA) formation occurs when free radicals attack the fatty acids in biological membranes, leading to structural and permeability changes. This may cause loss of selectivity during ion exchange, release of organelle contents, formation of cytotoxic products (such as malondialdhide), culminating in cell death .The observed protection may be related to the presence of that may act as hydrogen or electron donors as well as transition metal ion chelators ***Bastos et al., (2007b) and Yeh et al., (2008).*** These result are in the same line with ***Martins et al. (2009)*** who found that mice fed yerba mate had lower thiobarbituric acid reactive substances in the liver and suggesting that treatment with yerba mate extract protected unsaturated fatty acids from oxidation and hence protected the liver.

Administration of yerba mate tea caused an increase in the antioxidant enzymes activity in rats serum (GPX, SOD and CAT) and reduced glutathione (GSH); The antioxidant defense system is responsible for keeping the redox-active species under control. The primary defense system is composed of substances that prevent the generation or sequester ROS, thus blocking the initiation of the root chain. Antioxidant enzymes (CAT, SOD, and GPx) and non-enzymatic substances, such as reduced glutathione (GSH), are found in this system. The secondary defense system is composed of phenolic compounds, such as Chlorogenic acid, caffeine, phenolic acids and saponins .For these reasons; the regular ingestion of (MT) caused significant decrease in lipid peroxidation and increase the resistance of DNA to H2O2-induced DNA strand breaks in liver cells ***(Miranda et al ., 2008) (Gugliucci et al., 2009) and (Fillip et al., 2009).*** . In our study we observed the same result ; a significant increase in the modulation of the antioxidant enzymes

GPx, SOD, and CAT after 8 week of the oral administration of mate´ tea compared to control positive group .Mate extracts have been shown to inhibit LDL oxidation.

Our histopathological finding partially similar to those reported by ***Lidiane ,(2014)*** who mentioned that animals receiving the cafeteria diet showed moderate to severe swelling of hepatocytes, as well as poorly defined vacuoles, disorganization of hepatocyte cords and reduced capillary lumen. These signs were not diminished in the liver tissue of animals treated with aqueous extract of *I. paraguariensis* and cafeteria .The results concluded that ,drinking one to three cups of yerba mate tea improve the lipolytic activity

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تأثير مشروب شاى يربا متة فى تحسين وظائف بعض الإنزيمات الهاضمة للدهون على الفئران المصابة بالسمنة

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الملخص العربي

إستهدف هذا البحث التقييم الحسى للشاى المعد من الاوراق الجافة لنبات المتة البورغالى وفصل وتقدير المركبات الفينولية فى الشاى المعد ودراسة تا ثيره على الفئران المصابة بالبدانه تم الاختبار الحسى واجراء التحليل الكيميائى لفصل وتقدير المركبات البولى فينوليه فى الشاى المعد واوضحت النتائج ان الشاى المعد مقبولا حسيا وكانت اهم المركبات الفينوليه الموجوده به حمض الكلوروجين والكافيين وحمض الكافيك واهم الفلافونات روتين وكامفيرول وكوارشين والتى تم فصلها باستخدام جهاز كروماتوجرافيا السائل ذو الضغط المرتفع . وتم إجراء التجربة على عدد ٣٥ فأر قسمت إلى ٥ مجموعات كل منها ٧ فئران ، تركت إحداها مجموعة ضابطة سالبة .أما الاربع مجموعات الاخرى فتم تغذيتها على عليقة عالية فى محتواها من الدهون لمدة اربع اسابيع لاحداث البدانة بها . وتركت احدى المجموعات كمجموعة ضابطة موجبة وتم إعطاء الفئران البدينة مشروب الشاى على التوالى يوميا لمدة ثمانية أسابيع بثلاث جرعات عن طريق الفم . وتم وزن الفئران فى بداية التجربة ونهايتها وحساب الوزن المكتسب ومعدل التحويل الغذائى وفى نهاية فترة التجربة تم ذبح جميع الفئران وأخذ عينات من الدم لقياس مستوى ليبيدات وفوسفوليبيدات الدم وجلوكوز الدم و قياس وظائف كل من الكبد و الكلي ومستوى هرمونى الانسولين والليبتن فى الدم و كذلك قياس نشاط الانزيمات المضادة للأكسدة وانزيم ليبيز البنكرياس . وتم اجراء الفحص الهستوباثولوجى للكبد. وأظهرت نتائج الدراسة أن إعطاء الجرعة الكبيرة (٣مل/ ١٠٠جم من وزن الجسم) من الشاى المعد من الاوراق الجافة لنبا ت المتة للفئران المصابة بالبدانة ادى الى نقص معنوى فى مستوى إنزيمات الكبد، ،وظائف الكلى ،الكوليسترول ، الجلسريدات الثلاثية والكوليسترول منخفض الكثافةLDL-c والجلوكوز و نقص تركيز هرمون الليبتين ومستوى المالونالدهيد مقارنة بالمجموعة الضابطة الموجبة. وكذلك أدى إلى نقص تركيزات اليوريا وحمض البوليك فى السيرم وحدوث تثبيط لعمل انزيم ليبيز البنكرياس بينما أدى إلى زيادة فى محتوى الجلوتاثيون المختزل و نشاط الانزيمات المضادة للأكسدة و كذلك الكوليستيرول المرتفع الكثافة HDL-c و زيادة تركيز هرمون الانسولين . وأظهر الفحص الهستوباثولوجى للكبد اختفاء التغييرات الهستوباثولوجية فى الكبد فى الفران البدينة التى تم إعطائها الجرعة الكبيرة من الشاى المعد من اوراق نبات المتة وتوصى هذه الدراسة بان الشاى المعد من نبات المتة مقبولا حسيا وله تاثيرات تحسينية للمرضى الذين يعانون من البدانه وارتفاع مستوى السكر والدهون بالدم .