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Mimusops laurifolia leaves extract act as immunomodulator and decrease glycemic level in obese rats

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Abstract: This study evaluate the antioxidant potential of *Mimusops laurifolia* the phenolic and flavonoid content, as well as antioxidant activity at different extracts, were prepared as aqueous, ethanol, ethyl acetate, and chloroform. The results from the different extracts from *Mimusops laurifolia* reported that the phenolic and flavonoids content was the highest concentration in ethanol followed by ethyl acetate and chloroform, in addition, the aqueous extract was the lowest. Whilst, the antioxidant activity DPPH from *Mimusops laurifolia* indicated that the scavenging ability of different extracts may be dependent on the highest concentration of phenolic and flavonoid content in different extracts. The biological experimental was focused on characterizing the role of *Mimusops laurifolia* extract remarkably lipids profile, an antioxidant enzyme, immune-modulator, effective endogenous hypoglycemic shown through cortisol reduction and Glucose -6-phosphate dehydrogenase (G6PD) reactivity inhibition in obese male rats fed on a high-fat diet (HFD). At the end of the experiment, the obtained results found that improvement the lipids profile and antioxidant enzyme were taken orally/day at level 400mkg body weight /rats from *Mimusops laurifolia.* Moreover, it could be noticed that HFD supplementation exhibited a significant increase in the levels of serum IgG, IgM, Cortisol as well as the reactivity of G6PD whereas it exhibited a significant decrease in serum IgA level. From the previous results it could be recommended that the supplementation of *Mimusops. laurifolia* improved the lipid profile and antioxidant enzyme, and also, lowering glycemic and elevated immunoglobulinslevelsin obese rats**.**
[Ahmed Hussein Fadle-Alla Mohamed ***Mimusops laurifolia*** **leaves extract act as immunomodulator and decrease glycemic level in obese rats** *J Am Sci 2021;17(9):49-59]. ISSN 15451003 (print); ISSN 23757264 (online). http://www.jofamericanscience.org* 6.doi: 10.7537/marsjas170921.06.

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1. Introduction

Obesity is associated with chronic heart diseases, liver function disorder, hyper-diabetics, and cancers. The natural herbal like *Mimusops. Laurifolia* is widely utilized for obesity therapy(**Perumal *et al.*, 2021**).

Obesity is known as a condition of increased body mass index (BMI) and an excessive amount of body fat. It is associated with increased blood pressure, type 2 diabetes, cardiovascular diseases, and several types of cancer (**Lauby-Secretan, 2016)** such as liver, pancreas, colon, and rectum. The prevalence of overweight and obesity has increased drastically over the last few decades worldwide **(WHO, 2020).**

Currently, worldwide, there is an elevate in infectious diseases which demands efficient body defense tools to control them about the way of the immune system. Malnutrition and infectious diseases have highly compromised the body's immune system responses in humans, and also, reinforce one another synergistically **(Nfambi *et al.* 2015).** Therefore, the immunological processes are influenced by obtaining the food necessary for health and growth; therefore, there is a disorder imbalance among food intake and the specific immune responses mediated by cell-mediated mechanisms as appeared by increased activation of B lymphocytes in obese subjects and in type 2 diabetic patients **(Duffaut *et al.*, 2009).**

The High Fat Diet HFD-fed rats displayed a lowering in the number of the IgA in the macrophage subset, which has been linked to the regulation and expression of enzymes that induce the factors imprint gut homing of IgA B cells, as well as the synthesis and secretion of IgA to gut homing of IgA B cells, in addition to synthesis and secretion of IgA **(Kim*et al.*,2018).** Moreover, the reduction of IgA suggest that excessive fat intake increased modulating the adaptive mucosal immune connected with SIgA against gut microbial (**Teresia *et al.*, 2019).**

The increased lipogenesis and decreased lipolysis, together with a stimulation of hepatic gluconeogenesis and an inhibition of peripheral glucose utilization was associated with hyperinsulinism and excess of glucocorticoids (GCs). However, a consistent relationship between cortisol concentration and body mass index (BMI) is9.8% increase of cortisol for each 2.5-point increase in BMI **(Stalder *et al.*, 2017)**.

Limei *et al.* (2018) revealed that,mice fed a standard of HFD exhibited visceral fat accumulation, and insulin resistance coupled with

increased adipose tissue hexose-6-phosphate dehydrogenase (H6PDH) gene expression.

Jaffar *et al.* (2011) investigated the effect of ethanolic extract of the leaves of *Mimusops elengi* on carbohydrate metabolic enzymes activity in streptozotocin induced diabetic rats and showed a remarkable reduction of blood glucose levels in liver and kidney as well as the content of triglycerides, fatty acids, phospholipids, LDL and VLDL-cholesterol in the serum and tissues compared with controlled diabetic rats.

The objective of this study is to examine the biochemical effects at different levels of leaves extract of *Mimusops laurifolia* 100, 200, 33, and 400 ppm kg b.wt.) on humoral immunity response, glucocoticoids (cortisol) level, and G6PD activity in male rats stressed by HFD to compile evidence concerning its potential as an immunomodulator and hypoglycemic for developing novel therapeutic strategies in both research and medication fields.

2. Materials and Methods

Materials

The Leaves of *Mimusops laurifolia* were collected from the Egyptian museum Garden, Cairo, Egypt. The plant material was kindly identified by Dr. R. Hamdy, lecturer of plant taxonomy, Faculty of Science, Cairo University, Egypt. The leaves were dried, crushed, weighed, and stored in an air-tight bag at room temperature for further analysis.

Kits for determination of the parameters were purchased from Sigma-Aldrich Corp., MO, USA,

Male Wister albino weaning rats (60 rats) with weights ranging from 150-160g were purchased from National Organization for Drug and Control Research, Giza, Egypt.

Male Wister albino rats (60 rats) with weights ranging from 150-160g were purchased from National Organization For Drug and Control Research, Giza, Egypt.

Rats were housed in individual cages with screen bottoms and fed ad libitum on a basal diet for one week for acclimatization, which containing casein (20 %), corn oil (8%), corn starch (31%), sucrose (32%), cellulose (4%), salt mixture (4%) and vitamin mixture (1%) according to the method **Pell *et al.* (1992).**

Methods

Chemical composition and minerals content of *Mimusops laurifol* leaves

Chemical composition as moisture, crude protein, ether extract, ash, crude fiber, and carbohydrates was determined by using the methods of the **AOAC (2012).** Minerals content (Na, Ca, and K) were determined in the diluted solution of ash samples by using an emission flame photometer (Model Corning 410). The other minerals (Zn, P, Fe, and Mg) were determined by the Atomic absorption spectrophotometer (Perkin – Elmer Instrument Model 2380) were determined by using the methods of the **AOAC (2012).**

Preparation of leaves extracts

Dried powder of *Mimusops laurifolia* leaves (10 grams) was dispensed in 100ml of distilled water, ethanol, ethyl acetate and chloroform, overnight at room temperature using shaker. The mixture was filtered through what man No 1 filter paper and the extraction step was repeated twice. The filtrate was then concentrated to dryness at 40 °C ina rotary evaporator. The crude extracts were stored in a refrigerator until further analysis.

Estimation of total phenolic acids and flavonoids compounds of *Mimusops laurifolia* leaves extract:

The total phenolic content in the *Mimusops* leaves t extract was measured using the method of **Qawasmeh *et al.* (2012)** with Folin-Ciocalteu reagent. The UV reading was measured at 760 nm. Gallic acid was used as standard (1 mg/ml) and the results were expressed as gallic acid equivalent (GAE mg/100g of dry weight).

The total flavonoids content was determined by the method of **Eghdami and Sadeghi (2010).** The absorbance was measured against a blank solution at 510 nm and the total flavonoids content was expressed in terms of milligrams of quercetin equivalent (mg QE /100g DW).

Antioxidant activity

DPPH· (1,1-Diphenyl-2-picrylhydrazyl) Free radical scavenging assay

Determination of DPPH · free radical scavenging activity was measuredin green banana according to **Ravichandran *et al.* (2012**). The mixture was shakenvigorously and allowed to stand at room temperature. Butyl Hydroxy toluene (BHT, Sigma) was used as positive control while the negative control is contained the entire reaction reagent except the extracts. Then the absorbance was measured at 515 nm against blank.

The capacity to scavenge the DPPH · radical was calculated using the following equation:

DPPH · scavenging effect (Inhibition %) = [(Ac – As / Ac) × 100]

Where: Ac is the absorbance of the control reaction.

As is the absorbance in the presence of the plant extracts

Preparation of High-Fat Diet (HFD)

The high-fat diet (HFD) composition was adapted from **Levin and Dunn-Meynell (2002).**The HFD contains 414.0 kcal/100 g with carbohydrates (43%), protein (17%), and fat (40%). The diet is composed of 50% commercial food pellet, 20% milk powder (Dutch Lady), 24% ghee (Crispo), and 6% corn starch was fed to control positive and treated rats.

Biological experimental

Experimental rats were fed on a high-fat diet for 15 days and randomly divided into six groups ten rats for each. The 1st main group was fed on a basal diet for another 8 weeks and considered as control negative rats.

The other five rat groups with induction of obesity by feeding high-fat diet. were classified into control positive +(ve) as group (2), also, the rats of 3rd, 4th,5th, and 6th groups were fed separately on a high-fat diet and taken orally 100, 200, 300, and 400 mg/kg rat/ day of *Mimusops laurifolia* aqueous extrac bt for eight weeks.

At the end of the experiment, the blood samples were withdrawn from the orbital plexus, and centrifuged at 3000 r.p.m to obtain the sera after that, the sera were kept in a deep freezer at -20°C until their analysis.

Triglycerides, total cholesterol, HDL, and (LDL) were determined according to the method of **Fossati and Principe (1982), Allain *et al.* (1974), Lopes-Virella *et al.* (1977), and Steinberg (1981),** respectively. Moreover, serum glucose was determined according to **Tietz (1986).**

Oxidative stress as plasma Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GSH) were assayed by the method of**Aebi (1995)**, **Nishikimi *et al.* (1972), Paglia and Valentine (1967) and Factor *et al.* (1998),** respectively. The lipid peroxidation was determined colorimetrically as malondialdehyde (MDA) by **Yoshioka *et al.* (1979).**

Quantitation of IgG, IgA and IgM in serum were determined according to **Whicher *et al.* (1983)**, Cortisol was determined according to the method described by **Munro and Lasley(1988)**, G6PD activity was determined according to the method described by **Lohr and Waller(1974)** .

Statistical analysis

The statistical analysis of results were evaluated by computer software **SPSS** (ver. 22) using **ANOVA** with two factors of significance level when $p < 0.05$. Multiple comparisons were carried out applying LSD. Data were treated as complete randomization design according to **(Steel *et al.*, 1997)**.

3. Results and Discussion

Chemical composition and minerals content in *Mimusops laurifolia* leaves

The result in Table (1) showed that *Mimusops laurifolia* leaves contain moisture, crude protein, total lipids ash, crude fiber, and total carbohydrates were 10.21, 9.167, 2.28, 9.16, 13.69 and 67.25%, respectively. This implies *Mimusops laurifolia* leaves could be a good source of crude fiber, ash content and carbohydrates and it was lower in ash, crude fiber, and total lipids. The functional properties of food are associated with the content of fiber in the form of non-digestible carbohydrates, on which symbiotic bacteria feed in the large intestine **(Fuller *et al.*, 2016).**In people, dietary fiber is of great importance to the prevention and treatment of diabetes, obesity, coronary heart disease, as well as colon and large intestine cancers**(Brownlee, 2011).**

The same Table showed that the *Mimusops laurifolia* leaves had calcium, phosphorus, magnesium, potassium, iron, and Sodiumwere 4.52, 8.41, 0.28, 2.71, 0.83 and 7.72 ppm, respectively. From this result, it could be noticed that the *Mimusops laurifolia* leaves good source of phosphorus, Sodium, calcium, and potassium.

Calcium is a major factor sustaining strong bones and plays a part in muscle contraction and relaxation, blood clotting, and absorption of vitamin B12 potassium and magnesium are known to reduce blood pressure. Potassium plays a role in controlling skeletal muscle contraction and nerve impulse transmission. Patients with soft bone problems are usually placed on high calcium and potassium meals **(Kubmarawa *et al.*, 2007).** The iron content present in the extract can help in hemoglobin formation **(Latunde - dada, 1980)** and hence recommend for iron deficiency anemia. Various minerals are also co-enzymes in certain biochemical reactions in the body which underscores the importance of the plant in metabolic reactions.

Table (1): Chemicalanalysisandmineral content in *Mimusops laurifolia* leave son dry weight

Chemical analysis g/100g	***Mimusops laurifolia* leaves**	Minerals content ppm	***Mimusops laurifolia* leaves**
Moisture	10.21±0.83	Calcium	4.52±0.021
Protein	11.62±0.91	Phosphorus	8.41±0.042
Total Fat	3.741±0.02	Magnesium	0.28±0.01
Crude fiber	6.54±0.04	Potassium	2.71±0.013
Ash content	4.32±0.03	Iron	0.83±0.05
Total carbohydrates	77.00±6.24	Sodium	6.72±0.43

Values are mean and SD (n = 3)

Total phenolic and total flavonoids of *Mimusops laurifolia* leaves extracts

Table (1) showed that the determination of total phenolic and flavonoids in different extracts (aqueous, ethanol, ethyl acetate, and chloroform). From the results, it could be noticed that the total phenolic and flavonoids in *Mimusops laurifolia* leave at ethanol extract were the highest by 40.21 mg/100 GAE and 34.28 mg/100QE. These results confirmed by **Lapornik *et al.* (2005)** who showed that the ethanolic extract had contained the greatest phenolic and flavonoid contents were found from the cells membranes, of the ethanolic extract with high polarity to give increased polyphenol and antioxidant activity and inhibition the activity of polyphenol oxidase (PPO) which degrade the polyphenol in cells membranes **Turkmen *et al.* (2006).**

Moreover, in the same table, the results observed that the ethyl acetate extracted from *Mimusops laurifolia* leave had contained total phenolic and flavonoids were 29.83 mg/100 GAE and 22.36 mg/100 QE followed by chloroform extract was 20.59 mg/100 GAE and 14.28 mg/100 QE, respectively. Meanwhile, the aqueous extract from *Mimusops laurifolia* leave was the lowest in phenolic and flavonoids content. Polyphenolics had contained chemical compounds, like phenolic acids, flavonoids, stilbenes, and lignans **(Manach *et al.*, 2004).** These compounds are involved in plant defense mechanisms; in addition, it was health-promoting for the human. They act as antioxidants and thereby participate in the reduction of chronic diseases**(Huang and Shen, 2012).**Moreover, polyphenols are used to protect food from changes by microorganisms or by oxidation of fats **(Maqsood *et al.*, 2013).**

Table (1): Total phenolic acids and flavonoids compounds in of *Mimusops laurifolia* leaves extracts

Mimusops laurifolia Extracts	Total phenolic acids mg/100 GAE	Total flavonoids compounds mg/100 QE
Aqueous	8.35±0.21^{d}	6.94±0.15^{d}
Ethanol	40.21±2.31^{a}	34.28±2.15^{a}
Ethyl acetate	29.83±1.25^{b}	22.36±1.34^{b}
Chloroform	20.59±0.87^{s}	14.28±0.94^{c}

Values are mean and SD (n = 3); where: Mean values in the same with the letter are significantly different at p<0.05levels.

DPPH scavenging activity of *Mimusops laurifolia* leaves extracts at different concentrations

The results in Table (2) showed that greater DPPH scavenging activity from *Mimusops laurifolia* leaves from the ethanol, ethyl acetate, and chloroform extracts than the aqueous extract. The extracts with ethanol, ethyl acetate, and chloroform at a concentrate of 50µg/ml were 50.37, 45.28, and41.24%, respectively, followed by the aqueous extract was 41.68%. Moreover, the IC_{50} values were 40.38, 55.67, and 65.28, as well as in aqueous extract the IC50 was 85.24µg/ml, respectively, compared to BHT was 25.18µg/ml. It is better to mention that a lower IC50 value represents more potent free radical inhibitory activity. Thus, the present results indicated that ethanol, ethyl acetate, and chloroform extracts have powerful antioxidant activity followed by the aqueous extract. The strong antioxidant characteristics may be caused by the *Mimusops laurifolia* leaves extracts had contained the different antioxidant components **(Mrvcic *et al.*, 2012).** Moreover, **Sultana *et al.* (2007a,b)** found that the elevated activity of DPPH · radical scavenging activity may be caused by *Mimusops laurifolia* leaves had contained high amounts of total phenolic and flavonoids which are act as free radical inhibitors or scavengers, and acting probably as strong antioxidants.

Table (2): DPPH· scavenging activity of *Mimusops laurifolia* leaves extracts at different concentrations

Mimusops laurifolia Extracts	Scavenging activity %					IC_{50} µg/ml
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Aqueous	25.13±0.14^{e}	28.24±0.35^{e}	31.57±0.34^{e}	34.68±1.26^{e}	41.25±0.81^{e}	85.24±4.29^{a}
Ethanol	35.28±0.21^{b}	38.16±0.28^{b}	41.25±0.29^{b}	45.29±1.39^{b}	50.27±0.75^{b}	40.38±0.94^{d}
Ethyl acetate	32.15±0.23^{c}	33.27±0.34^{c}	36.62±0.31^{c}	40.38±0.67^{c}	45.28±0.43^{c}	55.67±1.61^{c}
Chloroform	28.27±0.27^{d}	31.49±0.26^{d}	34.12±0.28^{d}	37.29±0.91^{d}	41.24±0.90^{d}	65.28±2.38^{b}
BHT as standard	41.29±0.29^{a}	44.38±0.41^{a}	49.11±0.52^{a}	55.29±0.59^{a}	59.38±1.28^{a}	25.18±0.27^{e}

Values are mean and SD (n = 3); where: Mean values in the same with the letter ± are significantly different at p<0.05 levels

Effect of *Mimusops laurifolia* leaves extract on glucose and lipid profile in the rat group

Glucose and lipid profile were determined in the different obese at groups fed on high fat diet (HFD) and take *Mimusops laurifolia* leaves aqueous extract orally at 100, 200, 300 and 400 mg/kg rat/ day and the results are reported in Table (3).From the results, it could be noticed that the control positive the highest values of glucose, triglycerides, total cholesterol and LDL were270.0, 255.38, 210.18 and 140.28 mg/dl, and the lowest values in control negative in glucose, triglycerides, total cholesterol and LDL were 110.0, 121.52, 95.0 and 20.11 mg/dl, respectively. Whilst, the HDL was the lowest in control positive 23.21 mg/dl and the highest in control negative was 60.54 mg/dl. Moreover, the results from obesity different rat groups observed that the rat groups were taken the *Mimusops laurifolia* leaves aqueous extract to rally at 300 and 400 mg/kg rat/ day give the best results and nearly control negative. These results are similar to those shown that a high level of polyphenols in the different herbal may play a great significant role to the health benefit like lowering glucose, lipid profile, and chronic heart diseases **(Constantinou *et al*. 2008).** Different herb extracts had protection from chronic heart disease may be due to inhibition of LDL-C oxidation**(Sa, 2009).**The methanol (MeOH) extract from the leaves of different herbs were significantly lowering serum triglyceride (TG) in rats **(Ninomiya *et al.*, 2004).**

Several clinical and epidemiological studies showed that Hyperlipidemia is being a significant danger factor for chronic heart disease, is a dangerous public health trouble in the world **(Jaffer *et al.*, 2004).**Hyperlipidemia also has a vicarious function by activating the production of oxygen free radicals (OFRs) from polymer leukocytes (PMNLs) and monocytes **(Prasad, 2005).** Regarding is treatment, now a day there is an increasing interest towered the potential health benefits of medicinal plants like herbs.

Table (3): Fasting levels of some serum lipid patterns and glucose of negative control and obesity rats group after treated by *Mimusops laurifolia* leaves extract at the end of the study

Groups	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Glucose (mg/dl)
Negative control	**121.5±7.23^{e}**	**95.0 ±8..12^{e}**	**60.54±3.28^{a}**	**20.11 ±1.15^{e}**	**110.0 ± 7.36^{e}**
Positive control	**255.38±18.2^{a}**	**210.18±18.38^{a}**	**23.21±1.16^{e}**	**140.28±11.25^{a}**	**270.0 ± 15.27 ^{a}**
100mg/kg *Mimusops laurifolia*	**195.19 ±12.53^{b}**	**185.59±15.31^{b}**	**32.12 ±1.22^{d}**	**110.28±10.37^{b}**	**230.0 ± 12.38 ^{b}**
200mg/kg *Mimusops laurifolia*	**175.76±15.73^{c}**	**160.46±12.27^{c}**	**42.27 ±1.79^{c}**	**80.67± 5.39 ^{c}**	**190.0 ± 9.86^{c}**
300mg/kg *Mimusops laurifolia*	**145.64±11.29^{d}**	**130.39±9.87^{d}**	**50.38±1.67 ^{b}**	**50.73±4.15^{d}**	**150.0 ±7.38^{d}**
400mg/kg *Mimusops laurifolia*	**125.12 ±8.36^{e}**	**100.43 ±7.69^{e}**	**59.28±2.14^{a}**	**22.38±1.72^{e}**	**115.0 ± 6.35 ^{e}**

Values are mean and SD (n = 3); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Effect of *Mimusops laurifolia* leaves aqueous extract onantioxidant enzymesin the rat groups.

The results from Table (4) indicated that the antioxidant enzymes GSH, G-PX, and CAT were the lowest in the Positive control were 16.38, 10.76, and 4.29mmol/l, respectively, and the highest in MDA was 10.28 **nmol/l**. Meanwhile, the control healthy group the highest in the antioxidant enzymes was 102.12, 82.35, and 10.38 mmol/l, respectively, and the lowest in MDA was 0.50nmol/l.The different groups treated with *Mimusops laurifolia* leaves aqueous extract had the best results thus show the rat's group which taken orally 400mg/kg weight rat daily and fed on a high-fat diet gave 99.49, 80.36, and 10.11 mmol/l, and also, 0.72**n mol/l** in MDA, respectively.

These results administration of *Mimusops laurifolia* leaves extract at different levels caused an improvement in the activity of CAT enzymes than the negative control group. The development of CAT enzyme activity could be found in the antioxidant characteristics of *Mimusops laurifolia* leaves aqueous extract due to the presence of polyphenolic compounds that play a role in scavenging free radicals **(Rom *et al.*, 2016).** In addition, **Rouhi *et al.* (2017)** demonstrated that *Mimusops laurifolia* leaves aqueous extract at 400mg/kg weight rat was protected against lipid peroxidation due to maintenance of the GSH and serum levels and activities of CAT, GPx, and glutathione reeducates (GR) enzymes. The results from Lipid peroxidation as Malondialdehyde (MDA) in the same table were parallel and

confirmed the results from antioxidant enzymes. This was probably due to *Mimusops laurifolia* leaves aqueous extract has direct antioxidant effects or enhanced biosynthesis of GSH and the other antioxidant enzymes **(Ghanbarzadeh *et al.*, 2014).** In addition, *Mimusops laurifolia*leaves aqueous extractreduces the availability of lipids for peroxidation by transporting fatty acids into the mitochondria for β-oxidation and consequently mitigates the production and accumulation of lipid peroxidation products **(Derin *et al.*, 2006).** Moreover, the *Mimusops laurifolia* leaves aqueous extract reduced oxidative stress through attenuation of MDA production and improvement of the antioxidant status in testicular tissues via augmentation of SOD, CAT, GPx, and GSH levels. Our results were in harmony with earlier reports showing that *Mimusops laurifolia* leaves aqueous extract attenuated lipid peroxidation and enhanced the antioxidant balance in rat obesity **(Yuncu *et al.*, 2015).**

Table (4): Effect of *Mimusops laurifolia* leaves extract on antioxidant enzymes

Groups	GSH (m mol/l)	G- Px (m mol/l)	CAT (m mol/l)	MDA (n mol/l)
Negative control	**102.12±7.36^{a}**	**82.35±6.29^{a}**	**10.38±0.41^{a}**	**0.50±0.02^{f}**
Positive control	**16.38±1.38^{f}**	**10.76±0.82^{e}**	**4.29±0.07^{e}**	**10.28±0.82^{a}**
100mg/kg *Mimusops laurifolia*	**35.62±2.43^{e}**	**25.43±2.59^{d}**	**6.35±0.82^{d}**	**6.58±0.7d**
200mg/kg *Mimusops laurifolia*	**58.41±3.62^{d}**	**50.68±3.28^{c}**	**8.24±0.91^{c}**	**4.23±0.21^{c}**
300mg/kg *Mimusops laurifolia*	**75.39±5.19^{c}**	**65.28±4.83^{b}**	**9.76±0.98^{b}**	**2.490.08^{bc}**
400mg/kg *Mimusops laurifolia*	**99.49±6.38^{b}**	**80.36±6.83^{a}**	**10.11±0.89^{a}**	**0.72±0.06^{e}**

Values are mean and SD (n = 6); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Effect of *Mimusops laurifolia* leaves aqueous extract on serum Immunoglobulins

The obtained results in Table (5) showed that there is a significant increase in serum IgG, IgM while there is a significant decrease in serum IgA concentrations of rats supplemented with HFD as compared to the values recorded in healthy control rats and other groups administered extract only. These changes may be related to the local accumulation of B cells in the white adipose tissue (WAT)and increased production of pathogenic B-cell-derived IgG antibodies in the hypothalamic of HFD fed rats compared to the chow-fed control mice as stated by**Thaler *et al.*(2012)**. The obtained results for decreased IgA may be due to a significant shift in the frequency and number of IgA-producing plasma cells in the colonic lamina propria (LP).as stated by**(Helen *et al.*,2011)** who mentioned that, C57BL/6 mice fed on an HFD showed a large decrease in the secretory IgA and IgA-promoting immune mediators compared to normal diet-fed controls. Also, it could be found that increased IgM level remarkably in white adipose tissue of rats fed on HFD may be due to the circulating apoptosis inhibitor of macrophage (AIM) which interferes with the binding of IgM pentamers to Fc receptor for IgM and IgA antibodies, leading to abrogation of IgM from internalization through Fc α/μR and prolonged the presence of the IgM in the blood**(Kurokawa *et al.*, 2010)**.

The potential link between obesity and increased immunoglobulins production may be due to higher oxidation of lipoproteins yielding ox-LDL and MDA-LDL modified lipoproteins which are considered to be immunogenic therefore no longer be recognized by LDL receptors leading to the formation of pathogenic antibodies and immune complexes as recorded by **Song *et al.*(2014)**.

These findingsare also refered to the decreased gene expression assay that mediate plasma cell differentiation and decreased the gene encoding retinaldehyde dehydrogenase-1(Aldh1a-1) which is an important enzyme involved in the synthesis of retinoic acid (RA)essential for IgA production as stated by**(Seo *et al.*, 2013)** who recorded ,thatHFD-fed mice decreased secretory IgA (SIgA) concentrations within colon lumen and colonic.

The decreased production of immunoglobulin's may also attributed to *Mimusops* derived quercetin which regulate the immune response via enhancing NF-κB signaling pathway as well as increasing the secretion of IgA and interleukin-4 (IL-4) which plays a relevant role in B-cell class switching to IgE and decrease the production of Th1 cells which drive B cells for the production of immunoglobulin G2a (IgG2a) antibodies as supported by the findings of **(Yang *et al.*, 2019)**.

Table (5): Effect of *Mimusops laurifolia* leaves extract on serum Immunoglobulins parameters in rats' HFD-induced obesity.

Groups	IgG(mg/dl)	IgM(mg/dl)	IgA(mg/dl)
Negative control	323.00 ± 6.80^{a}	35.41 ± 1.35^{e}	4.07 ± 0.13^{d}
Positive control	195.10 ± 5.55^{e}	39.55 ± 1.30^{b}a	6.68 ± 0.11^{a}
100mg/kg *Mimusops laurifolia*	232.21 ± 2.93^{d}	38.40 ± 1.62^{b}	6.12 ± 0.18^{a}
200mg/kg *Mimusops laurifolia*	260.56 ± 6.51^{c}	37.08 ± 1.59^{c}	5.82 ± 0.14^{b}
300mg/kg *Mimusops laurifolia*	290.86 ± 5.34^{b}	36.66 ± 1.36^{d}	5.41 ± 0.08^{c}
400mg/kg *Mimusops laurifolia*	320.16 ± 5.54^{a}	35.38 ± 0.68^{e}	4.14 ± 0.09^{d}

Values are mean and SD (n = 6); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Effects of *Mimusops laurifolia* leaves extract on serum Cortisol and G6PDin rats obesity

Table (6) revealed that treatment with *Mimusops laurifolia* leaves extract to rats Supplemented by HFD significantly decreased levels of serum cortisol andG6PD enzymatic activity compered to the corresponding levelsin rats that supplemented by HFD only.

These results may be related to the presence of quercetin which is a potent suppressor to 11β-hydroxylase (CYP11B1) that catalyzes the final step of cortisol biosynthesis and secretionas confirmed by **Cheng and Li (2012)**.

Also plant containing quercetin may decrease cortisol level in female rats by decreasing oxytocin hormone that helps relaxation and reduction of blood pressure and cortisol levels as proved by **Matin and Ali(2016)**.

Variations in the results of cortisolmay be also due to the naturally occuring saponins which could target intracellular steroid hormone receptors in the kidney and act as a selective receptor antagonist to reduce the production of endogenous glucocorticoids mediated by these receptors and could reduce cholesterol contents, the raw material of steroidal hormones, by decreasing the expression of liver HMG-CoA reductase mRNA thus contributing to lower high cortisol level as stated by**(Siraj *et al.*, 2015**and**Liu *et al.*,2016)**.

The decreased G6PD activity in the results of our study may be relevant to the positive effects of *Mimusops* as anti-hyperglycemic and where deprivation of dietary carbohydrates essential to lipid metabolism has a specific effect on lipogenic liver G6PD formation reducing enzyme activity, maximum rate and catalytic efficiency due to inhibition of intracellular enzyme concentrations rather than changes in the activity of pre-existing enzyme which caused by absence of carbohydrate diet **(Jaffar *et al.*, 2011)**.

Possible inhibition of G6PDactivity may be thanks to the action of phenolic components found in plant materials which arrested mitotic clonal expansion in adipocytes of obesity-induced models and exerted inhibitory effect on associated ROS production via suppressing the expression of ROS generating genes such as G6PD and NOX4 and increasing ROS scavenging proteins such as SOD-1 and SOD-2 in adipocyte thus controlling the G6PD activity **(Jang *et al.*, 2019)**.

Table (6): Effects of *Mimusops laurifolia* leaves extract on serum Cortisol and G6PDin rats fed HFD-induced obesity.

Groups	Cortisol (µg/dl)	G6PD (U/g.Hb)
Negative control	7.71 ± 0.21^{a}	6.37 ± 0.30^{a}
Positive control	3.23 ± 0.13^{e}	2.84 ± 0.27^{e}
100mg/kg *Mimusops laurifolia*	4.59 ± 0.09^{d}	3.77 ± 0.23^{d}
200mg/kg *Mimusops laurifolia*	5.49 ± 2.14^{c}	4.20 ± 1.67^{c}
300mg/kg *Mimusops laurifolia*	6.47 ± 2.03^{b}	5.54 ± 0.55^{b}
400mg/kg *Mimusops laurifolia*	7.51 ± 1.80^{a}	6.85 ± 0.53^{a}

Values are mean and SD (n = 6); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Conclusion

From the above experimentation, it is apparently clear that manipulation of obesity in experimental models by *Mimusops laurifolia* leaves extract regarding its potential components may emerge a new therapeutic strategy based on their appreciable had contained antioxidant activity which improved lipid profile, an antioxidant enzyme, immunomodulatory activity, reducing excessive cortisol production in hypertrophic adipose tissue of these models and downregulating the increase of G6PD which may promote the development of T2D and CVD. An understanding of the multiplicity of pathways shared by obesity and *Mimusops laurifolia* leaves extract will inform the development of appropriate and strong prevention and intervention programs for treating the consequences of obesity and related metabolic disorders.

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