

# Lipid Lowering Potential of *Malakwang* (*Hibiscus*) Species Leaf Extract in Hyperlipidaemia-Induced Rats

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## Abstract

*Malakwang* (*Hibiscus* species) is a common vegetable regularly used in the diet and traditional health care support in Uganda. In this study, the efficacy of *malakwang* leaf extract as a potential regulator of serum lipids, urea and creatinine was investigated in hyperlipidemic rats. Forty two albino rats were arranged randomly into seven groups of six and fed with diets. Four experimental and three control groups were considered in the design. The rats in the experimental groups were fed on high fat diets containing different amounts of leaf extract from red and white *malakwang* variants. Control groups were fed on diets devoid of *malakwang*: a basic standard rat diet; high fat diet; and high fat with atorvastatin. The diets were administered daily and rat weight determined. On the last day, blood was drawn from the rats and the serum analysed for lipids, creatinine and urea using spectrophotometric techniques. Statistical analysis was used to estimate mean differences in weight and concentration of the biochemical parameters between experimental and control groups. Results showed decrease in weight gained up to week three and half in rats fed on the high fat diet with *malakwang* leaf extract. There was a significant difference in the levels of low density lipoprotein cholesterol ( $p < 0.05$ ), with lower levels in rats fed on 200 mg/kg red and 400 mg/kg white *malakwang* leaf extract. No significant change was noted in total cholesterol and triglycerides. Whereas there was a higher level of serum creatinine with the two *malakwang* variants ( $p < 0.05$ ), serum urea levels were significantly lower. Leaf extracts of both red and white *malakwang* (*Hibiscus*) exhibited capacity to reduce low density lipoprotein cholesterol, maintained serum urea but not creatinine. This may offer prospects for using *malakwang* in the dietary approaches to address public health concerns linked to high level of cholesterol.

## Keywords

*Malakwang* (*Hibiscus*), Cholesterol, Triglycerides, Urea, Creatinine, Atorvastatin

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## 1. Introduction

*Hibiscus* species plants have been excellent source of essential nutrients. Epidemiological studies have reported their wide range of remedies for metabolic syndromes including hypolipidemia, hypoazetomia, obesity, hypertension, diabetes, cancer, inflammation and the ability to regulate renal functions worldwide [1] [2] [3] [4] [5]. The therapeutic factors reported to be the attributing factors are the high content of vitamins and mineral elements as well as phytochemicals with antioxidants activities [6] [7]. The lipid lowering actions of *Hibiscus* species are mediated through inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine [8].

*Malakwang* (*Hibiscus* species) has a long history of use in food and traditional medicine in Uganda, making it an important potential medicinal plant. It is an indigenous and domesticated plant commonly grown in Northern, Eastern and part of Central regions of Uganda [9]. It is produced for food from the leaves and beverages from the seeds [10]. The two most commonly used variants of *malakwang* are the red and white. While the red and white variants are consumed or used to the same extent, they have inherent differences in taste and preference.

In Northern Uganda, of the few traditional plants used in the diet, *malakwang* is exceptional because of its sour bitter taste [10] [11]. Hot water or bicarbonate of soda is traditionally added to *malakwang* sauce to counter the acidic and bitter taste so as to improve palatability. *Malakwang* sauce blends well with roasted groundnuts/simsim paste. It also mixes well in other sauces like fermented hippopotamus skin, okra, beans, peas and fish. The seeds are roasted and ground to make a beverage which has similar flavour like coffee.

This work aimed at determining the weight, lipid, serum creatinine and urea lowering potential of *malakwang* (*Hibiscus*) species leaves and whether variants and concentration affect this property.

## 2. Materials and Methods

### 2.1. Selection of *Malakwang* Variants

The leaves of the red and white *malakwang* (*Hibiscus*) species variants were used in this study. The two plants are known traditionally by the local names; red for the plant with dull green leaves, faint red stalks and veins whereas the white for the plant with apple green leaves, green stalk and veins. The two *malakwang* samples were grown in Makerere University Agricultural Research Institute, Kabanyolo. The land had been fallowed for over three years. *Malakwang* leaves

for this study were harvested at six weeks old in the early morning hours by up-rooting the plants. The plants were packed in green perforated polythene bags and transported immediately to the laboratory. The two samples were then cleaned under running water to remove dust and debris present on the leaves and labelled. Thereafter, the leaves were plucked off the stalk; each sample was spread on separate clean tables to dry. The fresh leaves were measured in batches of 10 kg of each sample.

## 2.2. Preparation of Diets

Mice pellets procured from a local animal feed manufacturer—Engano Millers Uganda, were used as a basic standard rat diet. This contained cotton seed oil, ground sunflower seeds, silver fish, whole maize meal, maize bran, wheat bran, wheat pollard, oat, “vitamin and mineral premix” (Anupc Vitalyte, Anglian Nutrition Products, Suffolk, UK), powdered egg shell, dicalphosphate (DCP) and soya flour. The high fat diet was prepared from mice pellets (32.4%), lard (29.5%), sucrose (7.4%), milk powder (14.8%), margarine (7.4%), powdered egg yolk (4.4%), olive oil (2.9%) and 1.2% multivitamin (Anupc Vitalyte, Anglian Nutrition Products, Suffolk UK). The fat content of the high fat diet was 58.8%. Atorvastatin 10 mg, film-coated tablets (TEVA UK Ltd, East Bourne, UK) was used as a lipid lowering drug in the experiment.

## 2.3. Preparation of Extracts of *Malakwang* (*Hibiscus*) Species Leaves

The aqueous extracts of fresh leaves of both red and white *malakwang* were prepared using the procedures as described by Kate [12] and Gosain [13]. Total of 10 kg fresh leaves of *malakwang* was chopped and macerated using a blender, (Philips HR 2113 NL 9206 AD-4, Singapore). Distilled water at 60°C was added into the macerate mixed well with a wooden ladle, covered for 5 minutes and then filtered using one layer of fine muslin filter cloth. Little more water was added to rinse the residue after which it was filtered and added to the first filtrate. About 15,000 ml filtrate each of the white and red *malakwang* leaf variants were obtained. The filtrate was filtered again and then freeze dried using a high vacuum freeze dryer (Edwards High Vacuum, BOC Ltd Crawley Sussex, England). The product was packed and stored at 1°C. The dried leaf extract was used, at different concentrations, as feed component in subsequent experiments.

## 2.4. Experimental Animals

Wistar Albino rats supplied by the Pharmaceutical and Toxicology Laboratory, College of Veterinary Medicine and Bio-Security, Makerere University, Uganda were used. Forty two healthy adult rats (7 to 8 weeks old, and 65.5 to 143.5 g body weight) were selected for the study at a ratio of 1:1, male to female. They were housed in different cages based on randomly assigned experimental and control groups, and acclimatised for seven days while feeding them on basic

standard diet. Throughout the experiment period, the rats were maintained in a clean, well ventilated and quiet room. Fresh tap water from the animal laboratory was administered daily. The waste from the cages was removed daily. The rats were maintained at natural environment with room light regulated at 12 hours a day. The temperature of the room ranged between 20°C - 25°C night and day.

### 2.5. Experimental Design

The study was a randomized control experimental design. The forty two Wistar Albino rats were randomly set into seven groups of six rats each. Each of the seven groups was subjected to different diet treatments as follows:

Group 1 was fed on the basic standard rat diet (BSD).

Group 2 was fed on the high fat diet (HFD).

Group 3 was fed on high fat diet containing 200 mg/kg body weight of red *malakwang* leaf extract (MLE).

Group 4 was fed on high fat diet containing 400 mg/kg body weight of red *malakwang* leaf extract.

Group 5 was fed on high fat diet containing 200 mg/kg body weight of white *malakwang* leaf extract.

Group 6 was fed on high fat diet containing 400 mg/kg body weight of white *malakwang* leaf extract.

Group 7 was fed on high fat diet plus atorvastatin (by injection) at 5 mg/kg of body weight.

All feeds (basic standard and high fat diet) were administered orally at a rate of 20 - 25 g/rat/day and at the same time of the day. The extract of *malakwang* leaves in groups 3 - 6 was administered by oral-gastric tube feeding. Groups 1, 2, and 7 served as normal, negative and positive controls, respectively. The feeding experiment was done for four weeks. The compliance of the rats to the prescribed diets was monitored by taking records of the residues of the feeds daily before giving fresh feeds. The residue of diets was manually sorted out carefully from the waste and weighed (g). For the first five days from the start of the experiment, no residue was observed in the cages of the rats feeding on basic standard and high fat diets. All the rats were weighed weekly using electronic weighing scales (KDC/0.42A OHAUS Corporation, Pine Brook, NJ, USA).

### 3. Analysis of Biomarkers

On the 28<sup>th</sup> day of the experiment, the rats were anesthetized with mild chloroform (1 litre I.M.D.G. Code 6.1/111, UN. 1888IATA. 6.1Sd Fine chem. LTD., India) following the order of the experimental groups. After which about 10 ml of blood was drawn from each rat by heart puncture following standard procedure as described by Reddy [14] and collected in two sets of sterile standard blood bottles with anticoagulant and those without anticoagulant for biochemical analyses and stored under refrigeration. The bottles were labelled before putting

blood into them. Biochemical parameters included lipid profiles namely total cholesterol, triglyceride, high and low density lipoproteins and serum creatinine and urea. They were measured in an automated clinical chemistry analyser (Cobas 6000, Hitachi, Japan). Three hundred millilitres of sample blood was transferred into sample cup in the analyser and loaded into the rack which had the capacity of holding five samples at once. The rack was fitted into the track and the chemistry analyser was fed with reagents for each parameter and programmed to select the reagents and perform assessment [15]. The values of the parameters were read following spectrophotometric procedure and the results for individual rat were printed on individual sheet. At the end of each reaction, the sample cups were rinsed for the next measurement. When all the five samples were measured, the rack was unloaded and the next five samples were loaded and the procedures continued [15].

### 3.1. Histopathology Examination

Two rats in the ratio of 1:1 male to female were randomly sampled from each of the seven groups and used for histopathology analysis on assumption that the six rats in the groups were subjected to the same environmental conditions. The heart, liver, and kidney were carefully removed and weighed, then sectioned longitudinally into two halves and kept in 10% neutral formalin solution. Microscopic slides of the liver, kidney and heart were prepared where the organs were processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with haematoxylin and eosin and mounted onto microscope slides [14]. Organ samples were then examined for tissue fat deposition using Nikon florescent and normal light microscope (Eclipse Ci 5, Tokyo, Japan).

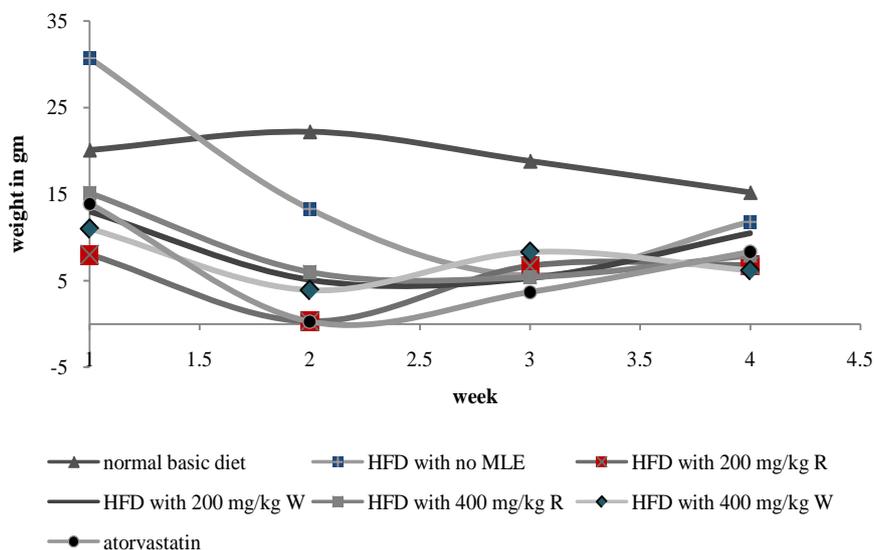
### 3.2. Statistical Analysis

The results were expressed as means, standard error of means (SEM). The significant differences among the groups were analysed using one way analysis of variance (ANOVA) followed by Students-Newman-kuels test for multiple comparisons among the groups. P value of 0.05 was considered statistically significant.

## 4. Results

Changes in weight gain were observed in Wistar rats fed on high fat diet and different dosages of *malakwang* (*Hibiscus*) species leaf extracts (MLE) for four weeks, (n = 6 animals) as indicated in **Figure 1**.

As shown, the body weight gain by the Wistar rats increased between the first and second week for the rats fed on basic standard diet; after which the weight gain reduced. For the rats fed on the high fat diet, the weight gained exhibited a decrease between the first week and third week after which it started to increase. Inclusion of both *malakwang* extract and atorvastatin in rats fed on



**Figure 1.** Weight gain in rats fed on different diets and extracts.

the high fat diet further decreased the weight gain up to the fourth week. The experimental groups demonstrated a significant weight reduction for a shorter interval of two weeks when compared to the rats fed on basic standard diet and high fat fed. The effect of *malakwang* extracts on weight gain, regardless of the concentration and variants of *malakwang* leaf was similar to that of atorvastatin fed rats although atorvastatin rats were faster in reducing weight in the first week. Different *malakwang* types and concentration exhibited similar weight gain trend.

#### 4.1. Serum Biochemical Profiles

The leaf extract of both red and white *malakwang* at all applied concentrations showed significant difference ( $p < 0.05$ ), in low-density-lipoprotein cholesterol (LDLC) when compared to the control groups fed on basic standard, high fat diet and atorvastatin fed rats (Table 1).

Treatment with *malakwang* leaf extracts had an effect of increasing serum creatinine while decreasing serum urea  $p < 0.05$  (Table 2). Use of atorvastatin and increased lipid content in the diet had no effect on the serum creatinine and urea levels compared to rats on basic standard diets  $p < 0.05$  (Table 2). The *malakwang* type and the extract concentrations exhibited no difference in reducing serum creatinine. Urea decreased with *malakwang* treatments when compared with the basic standard and atorvastatin induced rats. *Malakwang* extracts decreased the urea nitrogen-creatinine ratio.

#### 4.2. Histopathology Results

As indicated in Table 3 below, positive test indicating fat deposition in the rat organs were only noted in the heart of rats treated with 400 mg/kg of both red and white *malakwang* leaf extract.

**Table 1.** Serum lipid profiles of Wistar rats fed on high fat diet and different amounts of *malakwang* leaf extracts for four weeks.

Treatment	Mean (SEM) in millimoles/litre			
	Total cholesterol	Triglycerides	High density lipoprotein cholesterol	Low density lipoprotein
Basic standard diet (with no <i>malakwang</i> leaf extract)	2.0 <sup>a</sup> (0.1)	1.1 <sup>a</sup> (0.3)	1.6 <sup>ab</sup> *(0.1)	0.4 <sup>b</sup> (0.1)
High fat diet (with no <i>malakwang</i> leaf extract)	2.2 <sup>a</sup> (0.1)	1.6 <sup>a</sup> (0.2)	1.4 <sup>b</sup> (0.1)	0.4 <sup>b</sup> (0.1)
High fat diet with 200 mg/kg red <i>malakwang</i> leaf extract	2.3 <sup>a</sup> (0.1)	1.8 <sup>a</sup> (0.2)	1.8 <sup>a</sup> (0.1)	0.3 <sup>ab</sup> (0.0)
High fat diet with 200 mg/kg white <i>malakwang</i> leaf extract	2.1 <sup>a</sup> (0.2)	1.9 <sup>a</sup> (0.30)	1.6 <sup>ab</sup> (0.1)	0.3 <sup>ab</sup> (0.0)
High fat diet with 400 mg/kg red <i>malakwang</i> leaf extract	2.1 <sup>a</sup> (0.1)	1.7 <sup>a</sup> (0.1)	1.5 <sup>ab</sup> (0.2)	0.3 <sup>ab</sup> (0.0)
High fat diet with 400 mg/kg white <i>malakwang</i> leaf extract	2.0 <sup>a</sup> (0.1)	1.9 <sup>a</sup> (0.3)	1.4 <sup>b</sup> (0.1)	0.3 <sup>ab</sup> (0.2)
High fat diet and atorvastatin (fat lowering drug)	2.1 <sup>a</sup> (0.1)	1.9 <sup>a</sup> (0.4)	1.5 <sup>ab</sup> (0.1)	0.5 <sup>a</sup> (0.1)

\*Values in the same column sharing two letters superscripts are statistically different from the single letter superscripts at  $p < 0.05$ .

**Table 2.** Serum creatinine and urea profiles of rats fed on high fat diet at different concentrations of *malakwang* leaf extracts over four weeks.

Treatment	Parameter**	
	Creatinine ( $\mu$ moles/litre)	Urea (millimoles/litre)
Basic standard diet (with no <i>malakwang</i> leaf extract)	33.0 <sup>b</sup> (3.3)	6.5 <sup>a</sup> (0.5)
High fat diet (with no <i>malakwang</i> leaf extract)	35.5 <sup>b</sup> (4.2)	4.0 <sup>b</sup> (0.2)
High fat diet with 200 mg/kg red <i>malakwang</i> leaf extract	53.8 <sup>ab</sup> (1.8)	5.8 <sup>ab</sup> (0.6)
High fat diet with 200 mg/kg white <i>malakwang</i> leaf extract	44.8 <sup>ab</sup> (3.3)	4.7 <sup>ab</sup> (0.5)
High fat diet with 400 mg/kg red <i>malakwang</i> leaf extract	56.0 <sup>ab</sup> (3.6)	4.6 <sup>ab</sup> (0.3)
High fat diet with 400 mg/kg white <i>malakwang</i> leaf extract	44.0 <sup>ab</sup> (5.2)	4.5 <sup>ab</sup> (0.5)
High fat diet and atorvastatin (fat lowering drug)	38.5 <sup>b</sup> (2.3)	5.0 <sup>ab</sup> (0.6)

\*Values in the same column sharing two letters superscripts are statistically different at  $p < 0.05$  when compared with the control groups \*\*Values in parentheses are standard error of the mean.

## 5. Discussion

This study has shown that *malakwang* leaf extract, regardless of the concentration and variants, has the potential to reduce weight gain in a similar way to that of atorvastatin except that the rate of decrease by atorvastatin may be faster as indicated in the result. The decreased in weight gain of the experimental rats fed on *malakwang* (*Hibiscus*) species leaf extract together with high fat diet is in

**Table 3.** Fat deposition in the tissues of the liver, kidney and heart of Wistar rats fed on basic standard, atorvastatin, high fat diet and different amounts of extract of *malakwang* leaf for four weeks.

Treatment (n = 2)	Fat deposition in the organs		
	Liver	Kidney	Heart
Basic standard diet (with no <i>malakwang</i> leaf extract)	--	--	--
High fat diet (with no <i>malakwang</i> leaf extract)	--	--	--
High fat diet with 200 mg/kg red <i>malakwang</i> leaf extract	--	--	--
High fat diet with 200 mg/kg white <i>malakwang</i> leaf extract	--	--	--
High fat diet with 400 mg/kg red <i>malakwang</i> leaf extract	--	--	+
High fat diet with 400 mg/kg white <i>malakwang</i> leaf extract	--	--	+
High fat diet and atorvastatin (fat lowering drug)	--	--	--

+ values indicating fat deposition in tissues. -- values indicating no fat deposition in the tissues.

agreement with the report of Mohan [16] who fed Albino rats on high cholesterol diet together with extract of *Hibiscus cannabinus* leaves for four weeks and observed decreased in body weight. Preuss [17] fed volunteers 2000 Kcal diet together with hydroxycitric acid (a derivative of citric acid) daily for eight weeks and confirm the efficacy of hydroxycitric acid in weight management when the body weight of the volunteers decreased by 5.2%. Alaron [18] administered aqueous extract of *Hibiscus sabdariffa* containing 33.64 mg anthocyanin/120 mg extract to healthy obese mice orally for 60 days and noted significant weight reduction in the experimental mice. Huang [19] and Gosain [13] reported the same trend of decrease in weight in the subjects on treatment with extract from *Hibiscus sabdariffa* calices and leaves respectively. The reduction in weight (g) gain among the rats fed on different concentration of extracts could be attributed to the phytochemical and *Hibiscus* acids present in the extract of *malakwang* (*Hibiscus species*) leaves. Various scientific studies on extracts of *Hibiscus* leaf have reported that the chemical constituents responsible for weight reduction actions of leaves are phenolic compounds; anthocyanin, flavonoids and *Hibiscus* acids [16] [18] [19] [20]. The possible explanation for the reduction of weight gain effect of *malakwang* leaf extract could be due to the inhibition of adipocyte differentiation and adipogenesis by phenolic compounds present in *malakwang* leaf extract. Xiao [21] reported a novel antiadipogenic effect of *Sibiraea angustata* leaf water extract which impaired the proliferation adipo-differentiation of 3T3-L1 cells due to the inhibition of adipocyte differentiation and adipogenesis. Also *malakwang* leaf extract could have altered physiological processes or derangement of metabolic pathway of rats through the effect of saponins which prevents the absorption of lipid into blood. However the increase in weight after two weeks could be due to the possible result of the reduced weight gain which in turn activated adipocyte differentiation.

Whereas the experimental rats experienced weight reduction, the rats fed on

basic standard diet gained weight in the first two weeks. This is in agreement with the trend reported by Huang [19] who fed Hamster rats, standard laboratory diet in their experiment for 10 weeks and established evidence of weight gain. The exhibition of weight gain in the first week among the rats on basic standard diet could have been due to accommodation of excess lipids and glucose in the adipose tissues and muscle as a normal storage site in form of glycogen. Rosen [22] ascertained that adipogenesis occurs in rats when energy intake exceeds energy expenditure causing a positive energy balance. The decrease in weight gain after the first week could have been due to the effects of natural adipose metabolism regulatory mechanisms marked by the interplay between the opposing action of the hormones insulin and epinephrine and glucagon [22]. The later promotes mobilization of adipocytes causing morphological change, growth arrest and the production of hormones like leptin and adiponectin. Adiponectin helps in regulating glucose level as well as fatty acid breakdown [23]. Leptin regulates energy balance by inhibiting hunger homeostasis triggering reduction in consumption size [23]. Another important regulatory sensor of nutrients is sirtuin 1. Its role in adipose tissues is to protect them from inflammation and obesity under normal feeding conditions and foretell the progression to metabolic dysfunction [23]. These mechanisms could have controlled weight gain in the rats on basic standard diet despite the nutrition over load.

In a similar way the rat on high fat diet with no *malakwang* leaf extract exhibited decrease of weight gain up to three weeks, after which the weight started to increase (Table 1). The phenomenal of weight decrease could be attributed to several factors which are interrelated. The fully occupied glycogen stores, with continuous consumption of high fat diet could have caused severe expansion of adipose tissues associated with adipose inflammation and distorted adipokine profile consequently reducing consumption as a response to high leptin level [23] [24]. Also the effect of dysfunctional adipocytes which could have led to ectopic fat accumulation associated with insulin resistant might have caused lipotoxicity [24] [26]. In the context of insulin resistant/hyperglycaemia, the body cells do not get glucose from blood for energy, but burnt stored fat and muscle for energy causing reduction in overall body weight hence creating room for glycogenesis [25]. Another factor could have been the dietary stress signals induced by high fat diet that inhibited the activity of sirtuin 1 and caused gene expression changes [23] [24]. The altered metabolism improves  $\beta$ -cell function which in turn improves the endogenous insulin secretion that responds to lipid and glucose in blood from the high fat diet and further promotes unsaturated fatty acid synthesis [26]. These probably explain the increase, decrease of weight gain up to three weeks and the after change. It suggests that high fat diet may cause weight loss in a harmful way.

The lowering of low density lipoprotein cholesterol level shown in this study (Table 1) was the result of administration of red and white *malakwang* (*Hibiscus*) species leaf extracts at 200 and 400 mg/kg doses to Wistar rats. This obser-

vation is in line with the report by Gosain [13] who fed cholesterol induced Wistar rats different concentrations (100, 200, and 300 mg/kg) of extract of *Hibiscus sabdariffa* leaf orally for four weeks and noted significant reduction in low density lipoprotein (24% and 30%) but no significant change was noted in high density lipoprotein level. Decrease in low density lipoprotein cholesterol was also shown in hyperlipidaemia patients who were administered one 500 mg capsule of extract of *salvia Officinalis* leaf eight hourly for two months and experienced significantly decreased low density lipoprotein [27]. Reducing low density lipoprotein on feeding *malakwang* leaf extracts to rats revealed an important health potential of *malakwang* leaf extract demonstrated in this study. The lipid lowering effect of extracts of the red and white *malakwang* leaves on LDL-C can be attributed to polyphenols and antioxidants present in the extracts which could have modulated the activity of Hydroxymethylglutaryl co-enzyme A reductase. This could have contributed to upwards regulation of LDL-C receptor, consequently reducing serum LDL-C. Antioxidants and polyphenols found in the *malakwang* leaf extract include sitosterol- $\beta$ -D-galactoside, mucilage, saponins and probably *Hibiscus* acid. These substances could have been responsible for regulating low density lipoprotein cholesterol demonstrated in this study. Gosain [13] reported that sitosterol- $\beta$ -D-galactoside has the property to reduce the content of blood cholesterol and  $\beta$ -lipoproteins as well as normalising the cholesterol/phospholipids' ratio during hypercholesterolemia. Mucilage a soluble fibre forms a gel which traps cholesterol and expels it to the outside without passing it into the blood stream, consequently lowering the amount of cholesterol which enters the blood stream [28]. Gemede [28] further explained that mucilage helps to prevent the intestinal absorption of cholesterol produced by the bile for the digestion of food making mucilage anti-cholesterol. Another observation by Milgate [29] explained that saponins, which is glycoside compound binds with cholesterol and prevent its re-absorption into the blood stream resulting into its reduction in blood. In addition saponins causes resin-like action, thereby reducing the enterohepatic circulation of bile acids and increasing the conversion of cholesterol to bile in the liver resulting into lowering of serum cholesterol.

The low reducing effects of *malakwang* leaf extract on total cholesterol and triglycerides reported in this study when Wistar rats were fed on high fat diet and different amounts of leaf extracts of the red and white *malakwang* (*Hibiscus*) species agree with the findings reported by Kuiyan [3] whose experiment did not have a total cholesterol and high density lipoprotein blood lipid lowering effect. Alaron [18] also noted no change in triglycerides and total cholesterol from his experiment on obese mice. Studies conducted among human and rats have shown different results. Never the less, the increase level of serum triglycerides noted in this result could be due to the increase in mobilization of free fatty acids from the peripheral depots as reported by Mohammad [30]. Also the presence of antioxidant in *malakwang* leaf extract could have contributed to the

curbing of the enzyme needed to make triglyceride. The specific enzyme was most affectively reduced by the phenolic acid, o-coumaric acid and the flavonoids [31].

The kidney is imperative to total body homeostasis because it plays a principal role in the excretion of metabolic wastes and regulations of intracellular fluid volume, electrolytes composition and acid-base balance [1]. Therefore the ability of the kidney to clear serum creatinine and urea to normal level is an indication of healthy renal function. The elevated serum creatinine reported in this study occurred following the administration of leaf extract of the red and white *malakwang* at 200 mg and 400 mg/kg concentrations taken together with high fat diet. The finding is in agreement with the discovery reported by Saka [32] who fed 1 ml of Aloe Vera extract to male Sprague Dawley rats for 28 days and found significant increase in serum creatinine and urea. Another work by Madukwe [33] fed chows and fresh extract of herb leaf to rats and got significant increase in serum creatinine and BUN levels. Emelike and Dapper [1] fed albino rats different concentrations of aqueous extract of *Hibiscus sabdariffa* but evident no significant difference in serum BUN of the experimental groups. Mohagheghi [34] administered to hypertensive patients, 0.5% and 1% of extract of *Hibiscus sabdariffa* twice a day for 15 days but reported no significant effects on serum creatinine and BUN. The increase in serum creatinine evident in this study (Table 2) could be due to oxidative stress as a result of the long duration of feeding on high fat diet and extract of *malakwang* leaf on a daily bases causing accumulation of toxic in the kidney, electrolytes imbalance and acid-base balance and consequent impairment of muscle contraction [1] [33]. The control of serum urea level observed after feeding Wistar rats extracts of *malakwang* leaf and dietary fat, could be attributed to the activity of the phenolic glycoside factor which could have improved oxidative status in the kidney. Also saponins have been reported to impair the digestion of protein, affect growth and feed intake [33] [35]. These activities could have affected the level of blood protein and consequently deamination resulting into less production of urea.

The histopathology results illustrated the efficacy of the red and white *malakwang* (*Hibiscus*) leaf in the prevention of fat deposition in the liver, kidney and heart of rats over the four weeks in a dose dependant manner, just like atorvastatin has shown. It suggests that *malakwang* leaf extract is more effective in lower dose compared to higher dose of 400 mg/kg. This is in agreement with the report of Mohan [16] in which the histopathology of the liver showed no fat accumulation in the liver of hyperlipidemic rats. A similar effect of protecting the liver from fat deposition was reported by Bolkent [36] who fed animals with lipogenic diet and 2 g/kg daily with *Melissa officinalis* leaf extract for 28 days and the results indicated a reduction of lipid peroxidation in liver tissues. The physiological effects of *malakwang* on fat deposition could be attributed to phytochemical and possible antioxidant activities. Tannins as an antioxidant could have caused reduction in lipids peroxidation and prevented fat deposition in the

liver, kidney and heart among the experimental groups in this study. Tannins is acidic in reaction and was reported by Arvind and Alka [37] to have anti-oxidative property which is important in protecting cellular oxidative damage and lipid peroxidation, thereby offering protective effects to the organs. Another possible explanation is the antiadipogenic effect in 3T3 cells due to the inhabitation of adipocyte differentiation and adipogenesis contributed by antioxidant present in *malakwang* leaf extracts similar to the results reported by Kim [38], Xiao [21] and Harman [39] in their experiments. Dietary polyphenolics contain a number of phenolic hydroxyl groups which have beneficial effects mainly due to their mitochondrial reactive oxygen species scavenging activity which can provide protection against obese related liver and heart damages [22]. Treated animals with extract of *Hibiscus*, prevented low density lipoprotein cholesterol oxidation and modulated the production of monocyte chemo-attractant protein-1 [22]. Kao [40] treated oxidized low density lipoprotein mouse cell with *Hibiscus* extract (0.05 - 0.2 mg/kg) and largely prevented lipid accumulation in the heart. On the contrary, the 400 mg/kg concentration of *malakwang* leaf extract of the two variants did not seem to prevent fat accumulated in the heart of the rats. This could have been due to the effects of nutrition over load. The rats could have increased consumption as a result of improved appetite and reduced empty time of gastric intestinal tract. This occurrence supports the traditional claim that *malakwang* leaf is well known vegetable for improving appetite.

## 6. Conclusion

Base on the results reported in this study, the red and white *malakwang Hibiscus* species leaf extracts have constituted a consistent suppression of body weight gain in the Wistar rats over time. A noticeable potential value of the extract was demonstrated in regulating low density lipoprotein cholesterol and urea in Wistar rats. Further biochemical and molecular investigations with pharmacological and public health nutrition implications are recommended to establish conclusive findings.

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## Conflict of Interest

We declared no conflict of interest.

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