

## Analysing The Hypolipidemic Activities of The Tea Extracts of *Moringa oleifera*, *Glycyrrhiza glabra* and Their Blend at Different Concentrations, Orally Induced on Adult Male Wistar Rats

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**Abstract.** Herbal teas of *Moringa oleifera* (Moringa) and *Glycyrrhiza glabra* (Licorice) were known to have many beneficial effects, their possible hypolipidemic effects were investigated in this paper. Albino wistar rats were fed with aqueous extracts of *M. oleifera*, *G. glabra* and their blend teas (10, 30 and 50 mg/kg.BW/ml). The teas were found to lower the serum cholesterol, triglycerides, VLDL, LDL, and atherogenic index at their higher concentration, but were found to slightly increase the HDL as compared to the corresponding normal healthy rats fed with clean water (control). The phytochemicals screening of the teas aqueous extract were also investigated, the chemicals (tannins, saponins, flavonoids, terpenoids and glycosides) which were known to exhibit cholesterol lowering effect by inhibiting its absorption and simultaneous increase its excretion were found to be present in the teas extract. Thus, the study demonstrates and validates that *M. oleifera*, *G. glabra* and their blend possess hypolipidemic effect at higher concentration.

### Introduction

A diet high in saturated fat and cholesterol increases blood cholesterol and triglyceride levels. Hyperlipidemia is a collective term used to describe human conditions when a plasma level of one or more classes of lipids, namely cholesterol, triglycerides, phospholipids and fatty acids increases above normal levels. Hyperlipidemia is one of the major causes of the development of cardiovascular disorders. [20]

Almost all parts of the *Moringa oleifera* tree have been studied for several pharmacological actions. The aqueous extract of leaves of *M. oleifera* was reported for the wound healing [22] and antiurolithiasis activity [13]. The methanolic crude extract of *M. oleifera* shows antibacterial activity [19]. The bark extract has been shown to possess antifungal and antitubercular activity [3]. The ethanolic and aqueous extract of pods was found to be hypotensive [7].

Likewise, Licorice root (*Glycyrrhiza glabra*) was utilized in Chinese medicine and it was recommended as a cure for injury, for swelling, for its detoxification effect, and for improving health and lengthening one's life span [8]. It has been known that licorice has anti-inflammatory, anti-bacterial, antioxidative, anti-viral and expectorant properties [9, 27, 28] and is effective in the detoxification and protection of the liver [21]. Licorice extracts were found to have a wide range of biological activities, including phyto-components serving as antioxidants to fight against free radical-related diseases [21].

Plants are rich in a variety of compounds. Many are secondary metabolites and include aromatic substances, most of which are phenols or their oxygen substituted derivatives such as tannins [10, 11]. Many of these compounds have antioxidant properties. Peppermint (*Mentha piperita*), Licorice root (*Glycyrrhiza glabra*) and their blend are part of herbs taken in form of teas which are studied in this experiment.

In many cultures of the world, herbal remedies are increasingly being employed in an attempt to achieve the same purpose. Some researchers have validated the claim that, the leaves of these plants possess cholesterol-reducing effect and are used to treat patients with heart disease and obesity. However, this study has not been reported on "the processed teas" of *Moringa oleifera*,

*Glycyrrhiza glabra* and the blend of the two teas. For this reason it was decided to investigate and compare the effects of the aqueous extract of the teas of *Moringa oleifera*, *Glycyrrhiza glabra* and their blend on the lipid profile of the Wistar rat using experimental animal model.

## Material and Methods

### Sample Collection and Identification

Two different types of teas namely Licorice and Moringa were bought from the Tradomedical Centre, Ibadan, Oyo state, Nigeria.

### Chemicals

All chemicals and reagents were obtained from Sigma-Aldrich, USA and the analytical grade and kits were purchased from local distributors, Radox Laboratories Limited while the water used was glass distilled.

### Sample treatment and preparation

The tea extracts were prepared using hot water infusion. The mixture was filtered using No 1 Whatman filter paper and the filtrate kept prior analysis. The rats were weighed daily and the calculated volumes of the extracts in milliliters (mg/Kg.BW/ml) were administered orally for 30 days.

### Animals

Healthy adult male albino rats of Wistar strain weighing between 130 to 180 g were obtained from the animal house of the College of Agriculture, Federal University of Technology, Akure for the study. They were kept in rat cages at room temperature ( $27 \pm 2^\circ\text{C}$ ) and humidity ( $55 \pm 5\%$ ) and a 12 h cycle of light and dark. They were given free access to rat pellet and water *ad libitum*. The experiment was performed in accordance with the National Institute of Health guidelines of care and use of laboratory animals. The rats were allowed to acclimatize for a week before the experiment.

### Experimental design

There were 10 groups of 5 albino rats each, the experimental design were according to methods of [15] with slight modifications and it involved duration of 30 days.

1. Group 1: Control; group without treatment; normal diet and 0% of the tea samples
2. Group M1: 0.4ml aqueous extract of Moringa Tea; 10mg/kg BW/ml
3. Group M3: 0.4ml aqueous extract of Moringa Tea; 30mg/kg BW/ml
4. Group M5: 0.4ml aqueous extract of Moringa tea; 50mg/kg BW/ml
5. Group L1: 0.4ml aqueous extract of Licorice Tea; 10mg/kg BW/ml
6. Group L3: 0.4ml aqueous extract of Licorice Tea; 30mg/kg BW/ml
7. Group L5: 0.4ml aqueous extract of Licorice Tea; 50mg/kg BW/ml
8. Group M+L1: 0.4ml aqueous extract of Moringa+Licorice Tea; 10mg/kg BW/ml
9. Group M+L3: 0.4ml aqueous extract of Moringa+Licorice Tea; 30mg/kg BW/ml
10. Group M+L5: 0.4ml aqueous extract of Moringa+Licorice Tea; 50mg/kg BW/ml

### Blood collection and dissection

At the end of the experiment, blood was collected from each rat by cardiac puncture method. The blood was immediately transferred into appropriately labelled blood sample bottles containing anticoagulant and used to analyze the effect of the tea samples on biochemical lipid parameters such as triglycerides, HDL and total cholesterol.

### Phytochemical investigation of the extracts

The different chemical constituents present in aqueous extracts were subjected to the tests by Kokate [14] and Trease & Evans [30].

### Total Flavonoid content

The total flavonoid content of the extracts was determined using a slightly modified method reported by Chung *et al.*, [5]. Briefly, 0.5mL of enzyme digested sample was mixed with 0.5mL methanol, 50µl of 10% AlCl<sub>3</sub>, 50µl of 1mol L<sup>-1</sup> potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard by making use of a seven point standard curve (0, 20, 40, 60, 80,100 µg/mL). The total flavonoids content of samples was determined in triplicates and the results were expressed as mg quercetin equivalent per gram of the sample.

### Total Phenolic content

The total phenolic content of the samples extract was determined by the Folin-Ciocalteu assay as described by Chanda *et al* [4]. 500µl of Folin reagent was added and mixed with a solution containing 100µL of the extract and 2mL of distilled water. 1.5mL of 7.5% sodium carbonate was then added to the solution and the volume was made up to 10mL with distilled water. The mixture was left to stand for 2 h after addition of the sodium carbonate. The absorbance of the mixture was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). The standard used was tannic acid and the results were expressed as mg tannic acid equivalents per gram of the sample.

### Biochemical assays for lipids

Cholesterol, HDL and triglyceride levels were estimated from serum by using a commercial kit package (Randox Laboratories Limited). We used standard commercial kits for analysis as recommended by the manufacturer of these kits. LDL and VLDL-cholesterol were calculated following the method by Johnson *et al.* [12], while the atherogenic index was calculated by using the method described by Muruganandan *et al.* [17].

$$\text{LDL} = \text{TC} - (\text{HDL} + \text{Triglyceride}/5)$$

$$\text{VLDL} = \text{TC} - \text{HDL} - \text{LDL}$$

$$\text{Atherogenic index} = (\text{TC} - \text{HDL})/\text{HDL}$$

### Statistical analysis

Results are expressed as mean±SEM (standard error mean) and subjected to one-way analysis of variance (ANOVA) followed by Dunnett's test and values with  $p < 0.05$  were considered to be statistically different.

### Results and Discussions

Phytochemical investigation was performed and the following compounds were identified in the teas extracts as shown in Table 1. The phenolic and flavonoid contents of Moringa, Licorice and their blend vary as shown in figure 1 and 2. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts [31]. Phenolics and polyphenolics (polymeric phenolics) can provide relief from certain physical ailments and degenerative diseases in humans, including the reduction of cardiovascular disease and certain cancers [26, 1]. Therefore, it is not surprising that the extraction and analysis of phenolics from plants and other food sources have been extensively studied [18, 6].

**Table 1:** Phytochemical screening of the two tea extracts and their blends.

PHYTOCHEMICAL TESTS	M	LR	M+LR
ALKALOIDS	-	+	+
SAPONINS	++	-	+
TANNINS	+	-	+
PHLOBATAMINS	-	-	-
ANTHRAQUINONES	-	-	-
STEROIDS	-	-	-
TERPENOIDS	+	-	+
FLAVONOIDS	-	+	+
SALKOWSKI	-	-	-
CARDIAC GLYCOSIDES	+	+	+

**Key:** -- = Absent + = Present

**Table 2:** Effects of *Moringa oleifera* tea extract on blood lipid parameters (mg/dl) of the albino rats in different groups.

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	143.06±2.78 <sup>a</sup>	69.92 ± 0.75 <sup>a</sup>	43.54 ± 0.07	17.33±0.37 <sup>a</sup>	28.61±0.56 <sup>a</sup>	1.06±0.03 <sup>a</sup>
M1	134.72±1.39 <sup>a</sup>	148.87 ± 1.0 <sup>a</sup>	43.33 ± 1.09	78.59±0.36 <sup>a</sup>	26.94±0.28 <sup>a</sup>	2.44±0.06 <sup>a</sup>
M3	119.44±1.39 <sup>b</sup>	105.01 ± 0.75 <sup>b</sup>	44.35 ± 0.54	36.77±0.70 <sup>b</sup>	23.89±0.28 <sup>b</sup>	1.37±0.01 <sup>b</sup>
M5	114.58±0.69 <sup>c</sup>	80.70 ± 1.41 <sup>c</sup>	44.56 ± 0.41	13.23±0.46 <sup>c</sup>	22.92±0.14 <sup>c</sup>	0.81±0.006 <sup>c</sup>

Regular diet water *ad libitum* for control; values are expressed as mean±SEM, n=5; Data was analyzed by one way ANOVA followed by Dunnet test; Values with different superscripts in the same column differ significantly (P<0.05)

**Table 3:** Effects of *Glycyrrhiza glabra* tea extract on blood lipid parameters (mg/dl) of the albino rats in different groups.

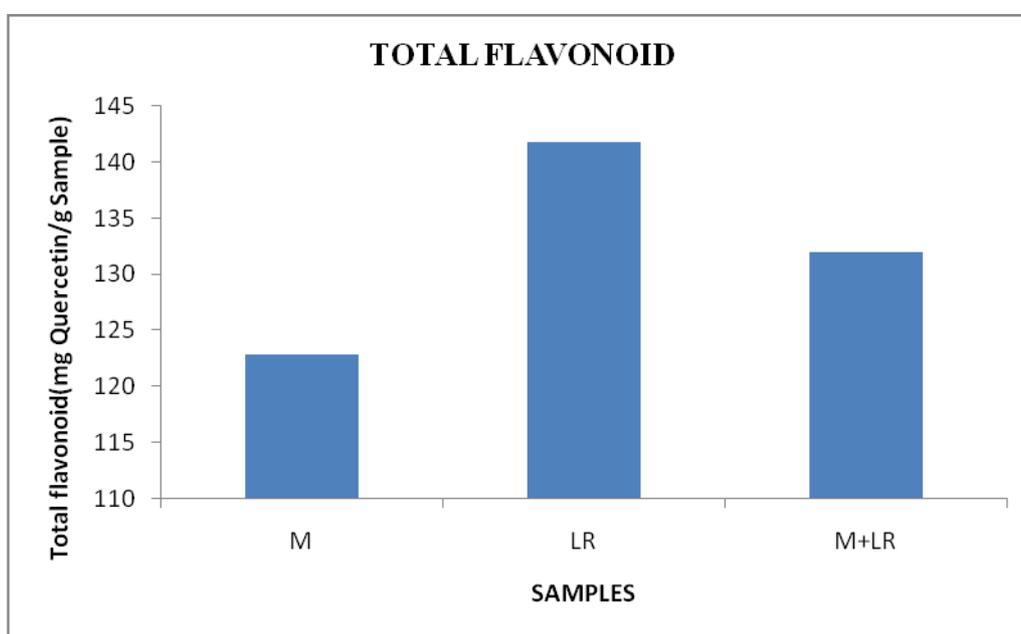
Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	143.06±2.78 <sup>a</sup>	69.92 ± 0.75 <sup>a</sup>	43.54 ± 0.07	17.33 ± 0.37 <sup>a</sup>	28.61±0.56 <sup>a</sup>	1.06±0.03 <sup>a</sup>
L1	172.22±1.39 <sup>a</sup>	139.85±0.71 <sup>a</sup>	44.69 ± 0.41	60.71 ± 1.19 <sup>a</sup>	34.44±0.27 <sup>a</sup>	2.13±0.039 <sup>a</sup>
L3	119.44±2.78 <sup>b</sup>	117.29±1.42 <sup>b</sup>	44.90 ± 0.34	48.51 ± 1.90**	23.89±0.56 <sup>b</sup>	1.61±0.042 <sup>b</sup>
L5	105.56±2.78 <sup>c</sup>	78.95±3.19 <sup>c</sup>	45.24 ± 0.20	12.60 ± 3.02 <sup>c</sup>	21.11±0.56 <sup>c</sup>	0.75±0.056 <sup>c</sup>

Regular diet water *ad libitum* for control; values are expressed as mean±SEM, n=5; Data was analyzed by one way ANOVA followed by Dunnet test; Values with different superscripts in the same column differ significantly (P<0.05)

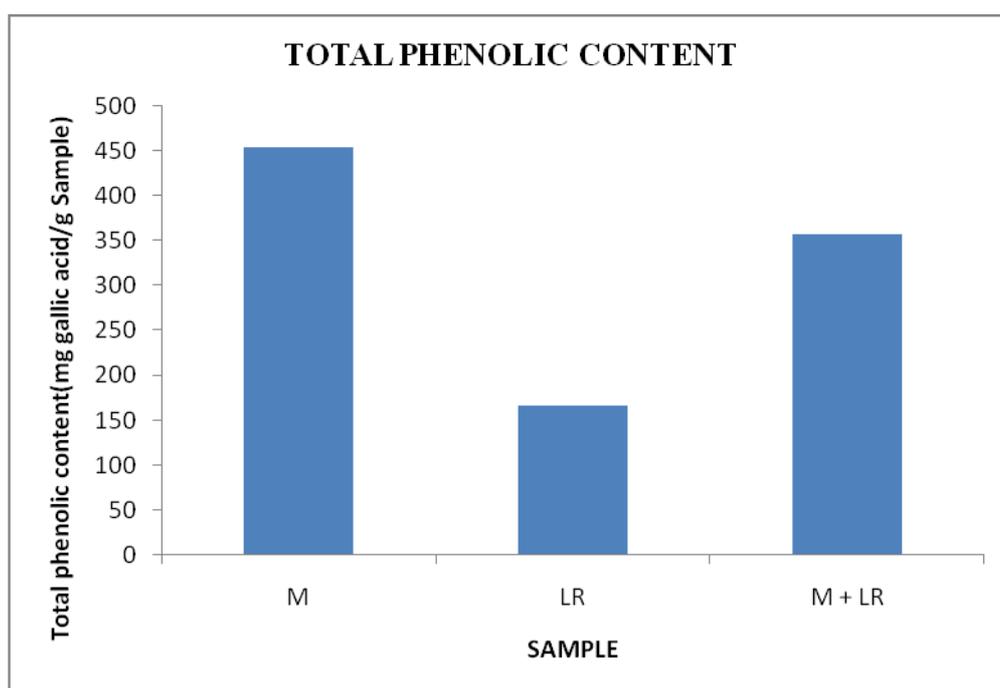
**Table 4:** Effects of the blends of *Moringa oleifera*, and *Glycyrrhiza glabra* teas extracts on blood lipid parameters (mg/dl) in different groups in albino rats

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	143.06±2.78 <sup>a</sup>	69.92±0.75 <sup>a</sup>	43.54±0.07	17.33± 0.37 <sup>a</sup>	28.61±0.56 <sup>a</sup>	1.06 ± 0.03 <sup>a</sup>
M+L1	155.56±2.78 <sup>a</sup>	152.13±1.06 <sup>a</sup>	45.78 ± 0.14	75.24 ± 1.17 <sup>a</sup>	31.11±0.56 <sup>a</sup>	2.32±0.006 <sup>a</sup>
M+L3	150.69±0.69 <sup>b</sup>	108.77±1.00 <sup>b</sup>	45.92± 0.20	32.71 ± 0.94 <sup>b</sup>	30.14±0.20 <sup>b</sup>	1.46±0.106 <sup>b</sup>
M+L5	129.17±0.28 <sup>c</sup>	102.01±3.90 <sup>b</sup>	45.99 ± 0.13	30.19 ± 2.07 <sup>b</sup>	25.83 ± 0.56 <sup>b</sup>	1.22±0.053 <sup>b</sup>

Regular diet water *ad libitum* for control; values are expressed as mean±SEM, n=5; Data was analyzed by one way ANOVA followed by Dunnet test; Values with different superscripts in the same column differ significantly (P<0.05)



**Figure 1:** Total Flavonoid content of Moringa tea, Licorice tea and their blend



**Figure 2:** Total Phenolic content of Moringa tea, Licorice tea and their blend

Flavonoids present in food of plant origin are also potential antioxidants [24]. Moringa, Licorice and their blend are good source of flavonoid (Figure 1). The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation [2].

Elevated level of blood cholesterol especially LDL-C is a known major risk factor for CHD whereas HDL-C is cardio protective. Treatment with aqueous extract of Moringa tea, Licorice tea and their blend, at three different doses, significantly decreased the levels of total cholesterol and LDL-C with respect to the normal control without tea extract [29] (Table 2, 3 and 4). This can be deduced from the results, as the concentration increases from 10mg/kg BW to 50mg/kg BW the values of total cholesterol decreases: for Moringa, from  $148.87 \pm 1.0^a$  to  $80.70 \pm 1.41^c$ , for Licorice from  $139.85 \pm 0.71^a$  to  $78.95 \pm 3.19^c$  and for their blend from  $152.13 \pm 1.06^a$  to  $102.01 \pm 3.90^b$  comparing with the control rats value  $69.92 \pm 0.75^a$ . Likewise LDL-C values decreases with the increase in extracts concentration: for Moringa, it decreases from  $78.59 \pm 0.36^a$  to  $13.23 \pm 0.46^c$ , for Licorice, it decreases from  $60.71 \pm 1.19^a$  to  $12.60 \pm 3.02^c$  and for their blend from  $75.24 \pm 1.17^a$  to  $30.19 \pm 2.07^b$ . Moringa and Licorice show a very significant decreases compare to control ( $17.33 \pm 0.37$ ) reinforcing their individual ability to lower LDL-C. The benefits and therapeutic significance of the two teas are visible in the average values of the blended sample as they exhibit the combination of the individual sample potential cholesterol-reducing ability.

Atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys. The higher the atherogenic index, the higher is the risk of the above organs for oxidative damage [16]. Atherogenic index was significantly reduced as the concentration of *M. oleifera* (from  $2.44 \pm .06^a$  to  $0.81 \pm .006^c$ ) and *Glycyrrhiza glabra* (from  $2.13 \pm 0.039^a$  to  $0.75 \pm 0.056^c$ ) increases. However, the blended groups (from  $2.32 \pm 0.006^a$  to  $1.22 \pm .053^b$ ) show a slight decrease in atherogenic index compared to control value ( $1.06 \pm .03^a$ ).

Rich fatty food causes the oxidative stress which does increases production of reactive oxygen species. A lot of scientific journals provides ample direct or indirect evidence that overproduction of ROS can induce cellular damage via oxidation of critical cellular components such as membrane lipids, proteins, and DNA. Since the result of the study indicated that the aqueous extracts of *M. oleifera*, *Glycyrrhiza glabra* and their blend, have beneficial effect on lipid profile, it will be good to identify what may be responsible for reducing potential of the teas.

Plant phytochemicals (such as tannins, glycosides, terpenoids, alkaloids, saponins, and flavonoids etc) inhibit the absorption of dietary cholesterol, but the resulting decrease in serum cholesterol has been slight [25]. Moringa tea has been shown to contain tannins, saponin, glycosides and terpenoid in phytochemical screening. Licorice tea has been shown to possess alkaloids, flavonoids and glycoside while the tea blend contains tannins, saponins, glycosides, flavonoids and terpenoid which are combination of the phytochemicals of the two teas (Table 1). The cholesterol lowering effect may be due to the inhibition in reabsorption of cholesterol from endogenous sources in association with a simultaneous increase in its excretion.

Conclusively, the observed cholesterol reducing action of the aqueous extract of *Moringa oleifera*, *Glycyrrhiza glabra* and their blend which may be responsible by their intrinsic phytochemicals, phenolic and flavonoid contents indicates their hypolipidemic activities.

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