

Effects of *Glycyrrhiza glabra*, *Mentha piperita* and their Blend Teas Infusion on Serum Lipids of Wistar Rats

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Abstract. The aim of this study was to demonstrate the effects of *Glycyrrhiza glabra* (Licorice), *Mentha piperita* (Peppermint) and their blend teas on serum lipids of Wistar rats. The animals were divided into control group (CG) and nine treated groups: P1, P3, P5 treated with peppermint tea; L1, L3, L5 treated with Licorice tea and P+L1, P+L3, P+L5 treated with blended peppermint and licorice tea of 10 mg/kg.BW/ml, 30 mg/kg.BW/ml and 50 mg/kg.BW/ml concentrations respectively. The teas were administered orally once in a day for 30 days. P3, P5; L3, L5 and P+L3, P+L5 animals showed significant decrease in triglycerides and total cholesterol and slight increase in HDL-c levels. The levels of LDL-c decreased in the treated groups compared to control group. The results suggested that supplementation with peppermint; licorice and their blend tea extracts can reduce the serum concentrations of cholesterol, triglycerides and LDL.

Introduction

A wide variety of plants are commonly used as remedies for the treatment of many diseases. The use of these plants to control blood lipid levels in plasma is widespread and many studies have shown that some plants do have beneficial effects [19, 27].

Low HDL-c (high density lipoprotein-cholesterol) and hypercholesterolemia levels are very common today and are often associated with endothelium dysfunction and inflammation, which are often accompanied by atherosclerosis. High levels of total cholesterol, LDL-c (low density lipoprotein cholesterol) and triglyceride associated with lower HDL-c levels can also induce higher risks of cardiovascular diseases [24, 25]. The combination of all these factors can trigger the Metabolic Syndrome [14, 9]. Endothelial dysfunction and cardiovascular diseases can be prevented by controlling serum lipids [22].

The lack of information about the possible toxic effects of many plants, allied to the high cost of allopathic This indicates the pressing need for new studies to medicines, encourages the use of the former in the treatment of many diseases. This indicates the pressing need for new studies to clarify the real effects of medicinal plants and to provide scientific evidence establishing their usefulness.

Licorice (*Glycyrrhiza glabra*) is one of the oriental herbal medicines that have been most frequently prescribed for the treatment of various diseases [23] and its extracts were found to have a wide range of biological activities, including antimicrobial, antiatherosclerotic, antihepatitis, antinephritic, and cardiovascular protective activities [7, 8].

Likewise, *Mentha piperita* is one of the ten most widely used plants in several countries [6] and is commonly used in the treatment of loss of appetite, common cold, bronchitis, fever, nausea, vomiting [2], spasmodic responses [15], and antimicrobial and antioxidant activities [16, 18].

Due to the important role of high plasma lipids in the development of insulin resistance, atherogenesis and cardiovascular disease, and the widespread use of *M. piperita* and *Glycyrrhiza glabra* as complementary and alternative medicinal intervention, this study aimed to investigate the effects of teas prepared from fresh peppermint and licorice processed teas (which is how this parts of the world where peppermint and licorice plants are not available, uses this plant) on Wistar rats plasma lipids.

Material and Methods

Animals

Healthy adult male albino rats of wistar strain weighing between 130 to 180 g were obtained from the animal house of the School 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 [13] 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 P1: 0.4ml aqueous extract of Peppermint Tea; 10mg/kg BW/ml
3. Group P3: 0.4ml aqueous extract of Peppermint Tea; 30mg/kg BW/ml
4. Group P5: 0.4ml aqueous extract of Peppermint 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 P+L1: 0.4ml aqueous extract of Peppermint +Licorice Tea; 10mg/kg BW/ml
9. Group P+L3: 0.4ml aqueous extract of Peppermint +Licorice Tea; 30mg/kg BW/ml
10. Group P+L5: 0.4ml aqueous extract of Peppermint +Licorice Tea; 50mg/kg BW/ml

Extract preparation and administration

Licorice and Peppermint teas were bought from the Tradomedical Centre, Ibadan, Oyo state, Nigeria. 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.

Phytochemical investigation of the extracts

The different chemical constituents present in aqueous extracts were subjected to the tests by Kokate [32] and Trease & Evans [31].

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, 50 μl of 10% AlCl_3 , 50 μl of 1mol L^{-1} 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 $\mu\text{g}/\text{mL}$). 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]. 500 μl of Folin reagent was added and mixed with a solution containing 100 μL 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 analysis

At the end of the experiment, blood was collected from each rat by cardiac puncture method. Blood samples were centrifuged (at 2000 g for 10 min); serum was obtained for the measurement of cholesterol, triglycerides, HDL by spectrophotometer 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 [10], 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 Discussion

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 Peppermint tea, Licorice and their blend vary as shown in figure 1 and 2. Peppermint and their blend showed the highest total flavonoid content (figure 1) and peppermint showed the highest phenolic content (figure 2). Tables 2, 3 and 4 show the effects of the peppermint, licorice and the blend of the two teas extracts on the blood lipid parameters of Wistar rats. The results showed that the different levels of peppermint, licorice and their blended extracts have no significant effects on serum HDL ($p > 0.05$). The serum contents of cholesterol, triglycerides, and LDL were significantly lower in the rats in groups P3, P5; L3, L5 and P+L3, P+L5 than in the control group ($p < 0.05$). The peppermint, licorice and their blended extracts at high levels had increased effects on the blood lipid parameters (P3, P5; L3, L5 and P+L3, P+L5 Vs P1; L1 and P+L1).

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

| Phytochemical Tests | P | LR | LR+P |
|---------------------|----|----|------|
| ALKALOIDS | ++ | + | ++ |
| SAPONINS | + | - | - |
| TANNINS | ++ | - | ++ |
| PHLOBATAMINS | - | - | - |
| ANTHRAQUINONES | - | - | - |
| STEROIDS | + | - | + |
| TERPENOIDS | + | - | - |
| FLAVONOIDS | - | + | - |
| CARDIAC GLYCOSIDES | + | + | + |

Key: + = Present - = Absent

Table 2: Effects of peppermint extract on blood lipid parameters (mg/dl) of the 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 ^a | 72.20±3.03 ^a | 43.54±0.07 | 17.33±0.37 ^a | 28.61±0.56 ^a | 1.06±0.03 ^a |
| P1 | 165.97±0.69 ^a | 116.79±0.50 ^a | 44.56±0.20 | 39.04±0.84 ^a | 33.19±0.14 ^a | 1.62±0.023 ^a |
| P3 | 64.58±7.64 ^b | 73.68±2.13 ^b | 44.90±0.27 | 15.87±3.30 ^b | 12.92±1.53 ^b | 0.64±0.043 ^b |
| P5 | 43.19±0.97 ^c | 57.89±0.75 ^c | 45.03±0.20 | 4.22±1.15 ^c | 8.64±0.19 ^c | 0.29±0.023 ^c |

Values with different superscripts in the same column differ significantly ($P < 0.05$). Values are expressed as mean \pm SE

Table 3: Effects of licorice extract on blood lipid parameters (mg/dl) of the 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 ^a | 72.20 ± 3.03 ^a | 43.54 ± 0.07 | 17.33±0.37 ^a | 28.61±0.56 ^a | 0.06 ± 0.03 ^a |
| L1 | 172.92±2.08 ^a | 139.60 ± 0.35 ^a | 44.69 ± 0.41 | 60.32±1.08 ^a | 34.72±0.28 ^a | 2.12±0.034 ^a |
| L3 | 118.75 ± 2.08 ^b | 117.29 ± 1.42 ^b | 44.90 ± 0.34 | 48.65±1.76 ^b | 23.75±0.42 ^b | 1.61±0.042 ^b |
| L5 | 104.31 ± 1.39 ^c | 69.17 ± 0.71 ^c | 45.00 ± 0.44 | 3.31±1.22 ^c | 20.86±0.28 ^c | 0.54±0.026 ^c |

Values with different superscripts in the same column differ significantly ($P < 0.05$). Values are expressed as mean \pm SE

Table 4: Effects of the blend of peppermint and licorice extract on blood lipid parameters (mg/dl) of the 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 ^a | 72.20 ± 3.03 ^a | 43.54 ± 0.07 | 17.33±0.37 ^a | 28.61±0.56 ^a | 1.06 ± 0.03 ^a |
| P+L1 | 136.11 ± 2.78 ^a | 114.29±1.50 ^a | 45.24 ± 0.20 | 41.83±1.86 ^a | 27.22±0.56 ^a | 1.53±0.022 ^a |
| P+L3 | 111.81 ± 0.69 ^b | 100.25±1.0 ^b | 45.44 ± 0.27 | 32.45±0.87 ^b | 22.36±0.14 ^b | 1.21±0.008 ^b |
| P+L5 | 83.33 ± 2.78 ^c | 83.46 ± 0.75 ^b | 45.78 ± 0.34 | 21.01±0.97 ^c | 16.67±0.56 ^c | 0.82±0.002 ^c |

Values with different superscripts in the same column differ significantly ($P < 0.05$). Values are expressed as mean \pm SE

Discussion

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 [28]. 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 [1, 30].

Flavonoids present in food of plant origin are also potential antioxidants [29]. Peppermint, Licorice and their blend are good source of flavonoid (Figure 1).

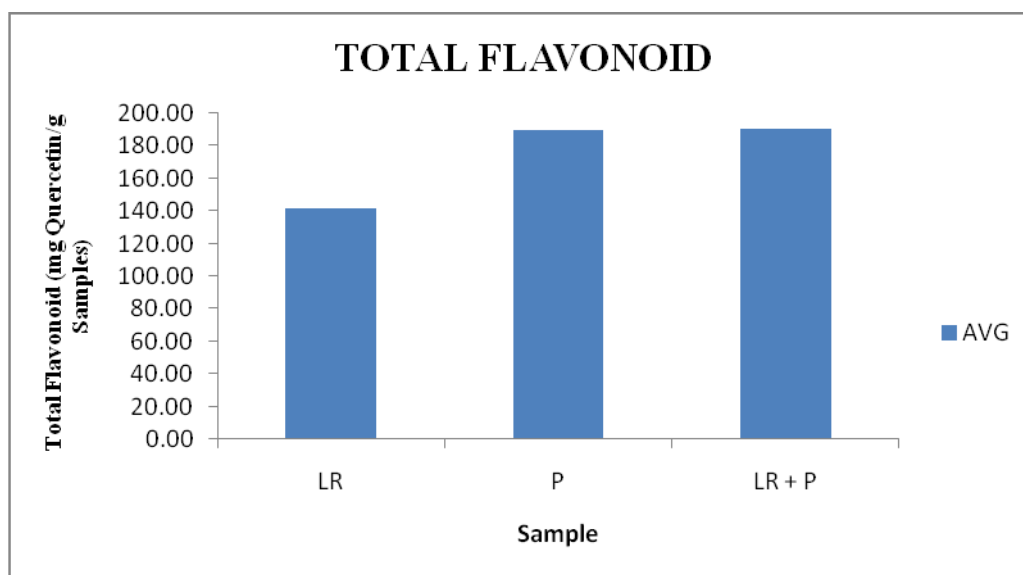


Figure 1: Total Flavonoid content of Licorice tea, Peppermint tea and their blend

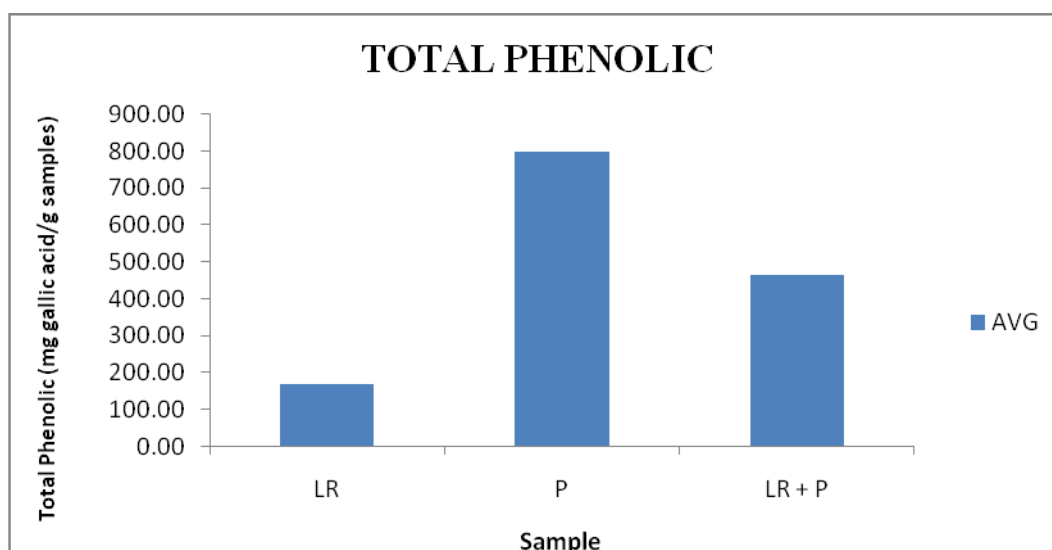


Figure 2: Total Phenolic content of Licorice tea, Peppermint tea and their blend

In this study, peppermint, licorice and their blend extracts did not have a significant impact on the serum content of HDL. The peppermint supplements had no significant effect on the serum content of HDL in broiler chicks [21]. The absence of concurrence among these studies may be a result of the levels at which the extract was administered. In addition, other variables, such as differences in background of the selected animals, the types of animals or genera and age may have affected the efficacy of extract usage, and therefore it was difficult to directly assess different studies that used extracts.

In this study, administering the peppermint licorice and their blend extracts at high levels decreased the serum content of LDL, triglycerides, and cholesterol in Wistar rats. It seemed that peppermint, licorice and their blend teas had anti-lipidemic benefits, but it cannot show this benefit at low levels. In relation to our findings, Johari et al. showed that peppermint extract decreased the serum contents of LDL, cholesterol, and triglycerides in Wistar rats compared with the control group [11]. The decrease of total cholesterol, LDL cholesterol, and total triglycerides of the treatment group might have been related to the involvement of the proposed components in the antioxidant and excess cholesterol protection mechanisms.

High temperature can induce the synthesis of free radicals, which can damage cell membranes by lipid peroxidation of polyunsaturated fatty acids in the cell membrane, thereby destroying the integrity of the membrane [14]. It seems antioxidants (peppermint, licorice and their

blend extracts) decrease lipid peroxidation in the plasma and tissues. The idea was confirmed by Young et al. who showed that herbs and spices, along with vitamins C and E (anti-oxidant vitamins) were even more effective at preventing lipid peroxidation in the tissues of birds [26]. There was a positive correlation between the total phenol concentration of herbal plants and human low-density lipoprotein oxidation in vitro [20]. I believe that the decrease in the serum content of cholesterol may be a result of inhibition of HMG-COA reductive activity, which is a key regulatory enzyme in cholesterol synthesis.

Conclusion

Peppermint, licorice and their blend tea extracts treatment did not improve the serum content of HDL. The results also suggested that supplementation with peppermint, licorice and their blend tea extracts can reduce the serum concentrations of cholesterol, triglycerides, LDL and VLDL and atherogenic index. This study showed that the individual of the samples extract is able to reduce the serum lipids, thereby helping to protect the rats against the harmful aftermath of lipoperoxidation and potentially ensuring antioxidant potential.

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