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The Hypolipidemic Activities of The Tea Extracts of St. John's Wort Tea, Chamomile Tea and Their Blend at Different Concentrations, Orally Induced on Adult Male Wistar Rats

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Abstract
St. John's Wort (*Hypericum perforatum)* and Chamomile (*Matricaria chamomilla*) are parts of well-documented medicinal plants in the world due to their many beneficial effects of which their possible anti-obesity effects were investigated by studying their influence on lipid profile. Albino Wistar rats were fed with aqueous extracts of St. John's Wort, Chamomile and their blend **teas** (10, 30 and 50 mg/kg.BW/ml). The teas were found to lower the serum cholesterol, triacylglyceride, 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 St. John's Wort, Chamomile and their blend possess anti-obesity effect at higher concentration.
Keyword: St. John's Wort , Chamomile, Phytochemicals, VLDL, HDL, LDL, serum

INTRODUCTION

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 **(Raida *et al*., 2008)**.

St. John's Wort (*Hypericum perforatum*) is an herbaceous perennial plant long known for its putative medical properties. St. John's Wort (*Hypericum perforatum*) extract (HPE) has been used for the treatment of neuralgia, fibrosis, depression and anxiety as an alternative to classic antidepressant **[11-13]**. St. John's Wort contains different groups of compounds such as hypericin, hyperforin and flavonoides. Hypericin and hyperforin are suggested to be responsible for its antidepressant effect **[11]**. However, there are few reports concerning the anti-obesity effect of HPE in rats.

In the same vein, Chamomile (*Matricaria chamomilla*) is one of the most widely used and well-documented medicinal plants in the world. Chamomile is also extensively consumed as a tea or tonic. Chamomile is used both internally and externally to treat an extensive list of conditions. It is used externally for ulcers, gout, eczema, skin irritations, sciatica, neuralgia, hemorrhoids, and rheumatic pain **(Newall et al., 1996)**. Also, the chamomile is the herb that is used for antioxidant agent, pain management, antispasmodic, anti-inflammatory, anti-convulsant, anti-pyretic, sedation, and wound healing in traditional medicine **(Namvaran-Abbas-Abad and Khayat-Nouri, 2011)**. The components of apigenin and Trihydroxyflavone in Chamomilla are glycosides that cause bitter taste. They also contain two important flavonoids called Pituitrin and Cyranosid **(Nouri and Abad, 2012)**. It seems that flavonoids are important in antispasmodic effects and main parts of the essence such as sesquiterpenes, chamazulene, α– Bisabolol and bisabolol have anti-inflammatory influences **(Baghalian et al., 2011)**.

In many tradition 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 carefully documented on "the processed **teas"** of *Hypericum perforatum, Matricaria chamomilla 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 *Hypericum perforatum, Matricaria chamomilla and their blend* on the lipid profile of the Wistar rat using experimental animal model.

MATERIAL AND METHOD

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 ± 2°C) and humidity (55 ± 5%) and a 12 h cycle of light and dark. They were given free access to rat pellet and water *ad labitum*. 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. There were 10 groups of 5 albino rats each, the experimental design were according to methods of **(Li *et al.*, 2004)** 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 ST1: 0.4ml aqueous extract of St. John's Wort Tea; 10mg/kg BW/ml
3. Group ST3: 0.4ml aqueous extract of St. John's Wort Tea; 30mg/kg BW/ml
4. Group ST5: 0.4ml aqueous extract of St. John's Wort Tea; 50mg/kg BW/ml
5. Group CT1: 0.4ml aqueous extract of Chamomile Tea; 10mg/kg BW/ml
6. Group CT3: 0.4ml aqueous extract of Chamomile Tea; 30mg/kg BW/ml
7. Group CT5: 0.4ml aqueous extract of Chamomile Tea; 50mg/kg BW/ml
8. Group ST+CT1: 0.4ml aqueous extract of St. John's Wort + Chamomile Tea; 10mg/kg BW/ml
9. Group ST+CT3: 0.4ml aqueous extract of St. John's Wort + Chamomile Tea; 30mg/kg BW/ml
10. Group ST+CT5**:** 0.4ml aqueous extract of St. John's Wort+Chamomile Tea; 50mg/kg BW/ml

Extract preparation and administration

St. John's Wort and Chamomile teas were bought from the Tradomedical Centre, Ibadan, Oyo state, Nigeria. The two teas were mixed to form blend sample by taking equal proportion of the teas. 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 extract

The different chemical constituents present in aqueous extracts were subjected to the tests by Kokate (1994) and Trease & Evans (1997).

Total Flavonoid content

The total flavonoid content of the extracts was determined using a slightly modified method reported by Chung *et al.,* (2002). Briefly, 0.5mL of enzyme digested sample was mixed with 0.5mL methanol, 50µl of 10% $AlCl_3$, 50µl 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 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* (2009). 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 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. (1997), while the atherogenic index was calculated by using the method described by Muruganandan et al. (2005).

LDL = TC – (HDL + Triglyceride/5)
VLDL = TC-HDL-LDL
Atherogenic index = (TC-HDL)/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 DISCUSSON

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 St. John's wort tea, Chamomile and their blend vary as shown in figure 1 and 2. St. John's wort showed the highest total flavonoid content (figure 1) and the highest phenolic content (figure 2). Plants consumed by humans may contain thousands of different phenolic compounds. The effects of dietary phenolics are of great current interest, due to their antioxidative activities. Flavonoids

present in food of plant origin are also potential antioxidants **(Satheeshkumar *et al*., 2011)**. 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 (**Benavente-Garcia *et al*., 1997)**.

High 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 St. John's Wort tea, Chamomile 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 (Table 2, 3 and 4). This can be deduced from the results of this study that as the concentration increases from 10mg/kg BW to 50mg/kg BW the values of total cholesterol decreases comparing with the control rats value. Likewise LDL-C values also decreases with the increase in extracts concentration. St. John's Wort and Chamomile showed a very significant influence on lipid profile compare to the control reinforcing their individual ability to lower LDL-C. The benefits and therapeutic significance of the two teas are visible in the average values of their blend sample as they exhibit the combination of the individual sample's 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 **(Mehta *et al*., 2003)**. Atherogenic index was significantly reduced as the concentration of St. John's Wort, Chamomile and the blended groups compared to the control value.

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 **(Saluja et al., 1978)**. St. John's Wort tea has been shown in this study to contain tannins, saponin, glycosides and terpenoid in phytochemical screening. St. John's Wort tea has been shown to possess alkaloids, flavonoids and glycoside while the teas 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 St. John's Wort tea, Chamomile tea and their blend which may be responsible by their intrinsic phytochemicals properties indicates the anti-obesity potentials of the teas.

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

PHYTOCHEMICAL TEST	ST	CT	ST+CT
ALKALOIDS	+	-	+
SAPONINS	+	-	+
TANNINS	+	+	+
PHLOBATAMINS	-	-	-
ANTHRAQUINONES	+	+	+
STEROIDS	+	+	+
TERPENOIDS	+	-	+
FLAVONOIDS	-	+	+
SALKOWSKI	+	-	+
Cardiac glycosides	++	-	+

Table 2: Effect of St. John's Wort on lipid profile of normal and experimental rats

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	157.43 ± 2.03 ^{a}	77.42 ± 0.65 ^{a}	40.64 ± 1.27	14.00± 0.71 ^{a}	31.49± 0.41 ^{a}	1.12 ± 0.04 ^{a}
ST1	281.08 ± 2.70 ^{a}	175.81 ±0.97 ^{a}	39.79 ± 1.06	39.07± 0.37 ^{a}	32.43± 0.27 ^{a}	1.80± 0.05 ^{a}
ST3	260.81 ± 2.70 ^{b}	99.03 ± 1.61 ^{b}	40.85 ± 0.21	26.69± 0.99 ^{b}	31.49± 0.41 ^{b}	1.42± 0.02 ^{b}
ST5	146.62 ± 3.38 ^{c}	57.74 ± 0.32 ^{c}	41.49 ± 0.21	15.44± 0.10 ^{c}	30.81± 0.54 ^{c}	1.11± 0.005 ^{c}

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

Table 3: Effect of Chamomile on lipid profile of normal and experimental rats

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	157.43±2.03[a]	77.42 ± 0.65[b]	40.64 ± 1.27	14.00± 0.71[a]	31.49± 0.41[a]	1.12 ± 0.04[a]
CT1	209.46±4.05[a]	22.26 ± 1.61[a]	39.59 ± 1.06	31.24± 0.11[a]	33.38± 0.41[a]	1.63± 0.03[a]
CT3	162.16 ±2.70[b]	13.55 ± 0.65[b]	40.21 ± 0.85	24.11± 0.14[b]	31.49± 0.14[b]	1.38± 0.01[b]
CT5	111.49 ±4.73[c]	6.13 ±0.32[c]	41.70 ± 1.06	14.66± 0.63[c]	26.22± 0.27[c]	0.98± 0.01[c]

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

Table 4: Effect of St. John's Wort and Chamomile blend tea sample on lipid profile of normal and experimental rats

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherogenic index
Control (No Teas)	157.43 ± 2.03[a]	77.42 ± 0.65[a]	40.64 ± 1.27	14.00± 0.71[a]	31.49± 0.41[a]	1.12 ± 0.04[a]
ST+CT1	288.51 ± 2.03[a]	82.90 ± 0.46[a]	39.15 ± 0.21	15.73± 1.25[a]	57.70± 0.41[a]	1.88± 0.04[a]
ST+CT3	181.08 ± 1.3[b]	66.45 ±1.29[b]	39.47 ± 0.31	15.93± 1.64[b]	36.22± 0.27[b]	1.32± 0.03[b]
ST+CT5	153.38 ± 2.03[c]	49.03 ± 2.58[c]	41.38 ± 0.53	5.68± 1.35[c]	30.68± 0.41[c]	0.88± 0.001[c]

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

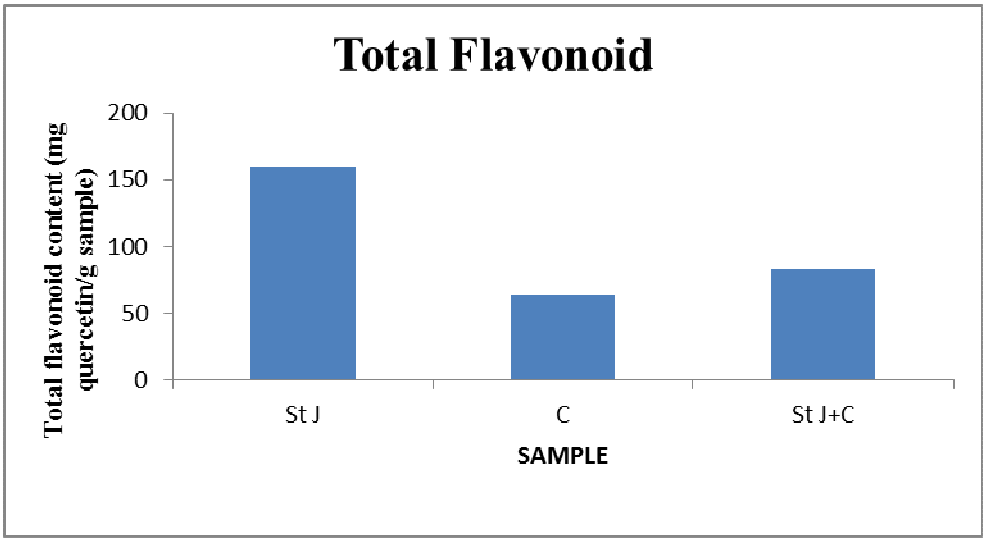

Figure 1: Total Flavonoid content of St. John's Wort , Chamomile and their blend

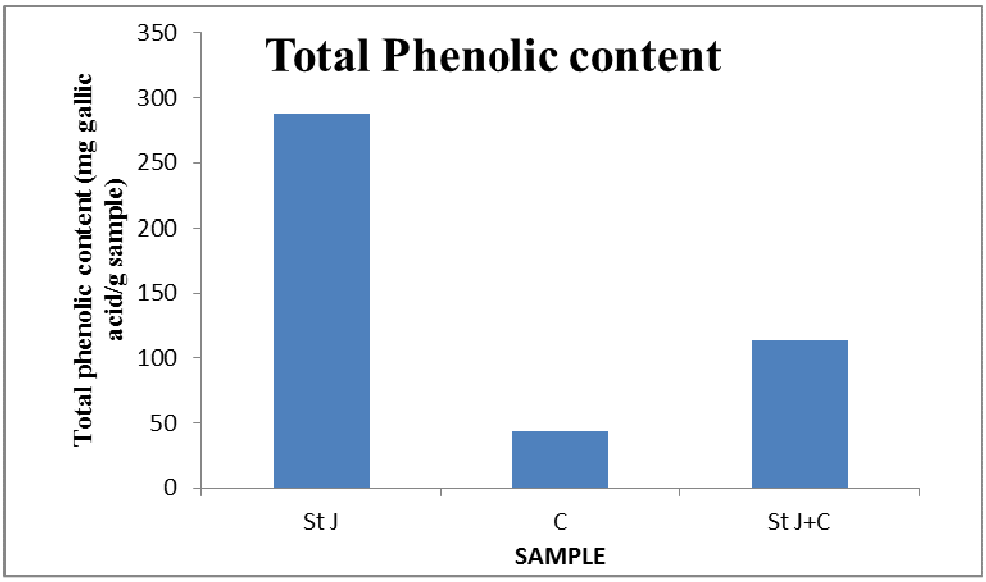

Figure 2: Total Phenolic content of St. John's Wort , Chamomile and their blend

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