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Safety Evaluation of 60-Days Ingestion of Statroltea: A Herbal Tea Beverage from the Leaves of *Stathmostelma* sp.

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Abstract

The safety of the sub-chronic ingestion of Statroltea, an anti-obesity herbal tea, was assessed in male rats fed either a standard diet or a high-fat diet associated to its daily administration at 5 mg/Kg BW during 60 days. Results revealed that the consumption of Statroltea significantly increases white blood cells count in male rats and produces a significant decrease in the relative weight of kidneys and liver of rats ($p < 0.05$). The administration of Statroltea did not change the activity of the hepatospecific enzyme alanine aminotransferase or the level of creatinine in serum irrespectively of diet. This is the first report on the safety of Statroltea and the study reveals no adverse effects linked to its consumption.

***Keywords**: Safety, Statroltea, herbal tea, 60 days – ingestion*

1. Introduction

Owing to the adverse side effects associated with many synthetic drugs, more recent trials have focused on screening herbal sources that have been reported to improve health with minimal side effects (Kishino *et al.*, 2006). In the northern regions of Cameroon, Statroltea, a phenolic- rich herbal tea beverage obtained from the leaves of *Stathmostelma sp.* is frequently consumed by the natives to reduce obesity and hyperlipidemia. Feumba *et al.* (2014) reported that Statroltea improve lipid profile by reducing blood levels of triglycerides, total and LDL cholesterol. However, no work has been reported on its adverse effects. It is against this background that the study aims to investigate the safety of Statroltea.

2. Main body

2.1. Production of Statroltea

The fresh leaves of *Stathmostelma sp*. were harvested in Ngaoundere, Cameroon and identified at the Cameroonian National Herbarium (Voucher N° 59014). Leaves were roasted at 144 °C for 20 min and cooled to room temperature. Roasted leaves were ground and bagged in 3 grams. Bags containing 3g of roasted leaves were brewed for 23 min in 300 mL of pre-heated water at 60 °C to give an infusion called in this study Statroltea. Statroltea was dried at 40 °C to obtain a dry residue that was used in the study. The composition of Statroltea expressed in percentage of total soluble solids (TSS) is recorded in Table 1.

***Table 1.** Basic proximate and phenolic composition of Statroltea*

Nutrients	**(mg% TSS)**
Total free sugars	349.68 ± 6.09
Reducing free sugars	330.57 ± 5.56
Crude proteins	666.87 ± 21.09
Phenolic compounds	**(mg% TSS)**
Total Phenolics	97.64 ± 1.35
Total flavonoids	68.10 ± 3.59
Total tannins	16.17 ± 0.41

Values represent mean ± SD of 3 replicates; Total phenolics were expressed as mg gallic acid equivalents % TSS; total flavonoids were expressed as mg quercetin equivalents % TSS; Total tannins were expressed as tannic acid equivalents % TSS; Total free sugars and reducing free sugars were expressed as fructose equivalents % TSS; Crude proteins were expressed as N x 6.25

2.2. Experimental design

2.2.1. Experimental Animals

Male Wistar rats, aged 3–4 months, weighing 250-350 g, were purchased from the animal house of the Laboratory of Animal Physiology, Faculty of Sciences of the University of Ngaoundere, Cameroon. Animals were maintained in standard laboratory conditions (23 ± 2°C, 12 h photoperiod) having free access to tap water and food. Rats were weighed and examined for physical abnormalities a day before initiation of test. The experimentation was conducted during 60 days during which animals were fed either with high-fat diet (10 rats) or with standard diet (10 rats). The standard and the high-fat diets were formulated as reported in table 2. Procedures used in the study were approved by the Animal Ethics Committee of the Ngaoundere University.

Table 2. Composition of standard and high-fat diets

Ingredients (%)	Standard diet	High-fat diet
Cassava starch	69.5	39.5
Casein	21.0	21.0
Soy bean oil	5.0	5.0
Palm oil	0.0	30.0
Vitamin mix	1.0	1.0
Mineral mix	3.5	3.5

2.2.2. Sub-chronic administration of Statroltea

For each diet, rats housed individually received distilled water (5 control rats) while treated groups (5 animals) received Statroltea at the dose of 5 mg/kg. Every day, a mass of Staroltea (1.25-1.75 mg) corresponding to the dose of 5 mg/Kg of body weight was dissolved in 5 mL of distilled water. The tea solution was mixed with 3 g of appropriate meal and the tea treated-meal was given to each animal. Rats were given *ad libitum* portion of untreated standard or high-fat meal after they have completely eaten the tea-treated meal. Statrotea was administrated during 60 days.

2.3. Evaluation of the relative weight of organs

At the end of the treatment, all animals fasted overnight and were anesthetized afterwards for blood collection from the jugular vein. After blood collection, all animals were immediately sacrified for gross pathological examination of the internal organs. The organs such as heart, lungs, livers, kidneys, brain and stomach were removed, blotted free of blood and weighed immediately. These organs were collected, weighed to determine relative organ weights.

2.4. Measurement of hematological parameters

Blood samples were collected into EDTA tubes for hematological analysis using an autohematology analyzer BC-3000 plus (Schenzhen, Mindrey). Hematological parameters measured were white blood cell (WBC), red blood cell (RBC), hemoglobin, hematocrit (Hct), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet, mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW).

2.5. Measurement of biochemical parameters

Blood samples were collected into dry centrifuge tubes and the blood was allowed to coagulate before being centrifuged at 3000 rpm for 10 min. The serum was separated and stored at −20 °C until the determination of biochemical parameters of rats.

Liver functions were assessed by evaluating the activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and the levels of total protein and albumin using commercial enzymatic kits (Randox, UK).

Renal function indices assay in serum was assessed by determining the concentration of creatinine, uric acid using commercial enzymatic kits (Randox, UK) and the level of some electrolytes using flame absorption spectroscopy.

3. Results and Discussion

3.1. Effect of 60-days administration of Statroltea on hematological parameters

The analysis of blood parameters is relevant in risk evaluation as the changes in the hematological system have a higher predictive value for human toxicity, when the data is translated from animal studies. Table 3 reveals the effect of Statroltea on hematology in rats. Subchronic administration of Statroltea did not cause any significant change in hematogical profile except WBC count which increased significantly ($P < 0.05$) in the treated groups compared to the controls. This increase was irrespective of the diet (table 3). An increase in WBC directly indicates the strengthening of the organism defense (Chang-Gue *et al.*, 2003; Stanley *et al.*, 2005). This elevation in total leukocytes count suggests that Statroltea contains bioactive compounds that have the ability to boost the immune system through increasing the population of defensive WBC.

Table 3: Effect of Statroltea on hematological elements of male rats

Hematological parameters	Standard diet		High- fat diet	
	Control	Statroltea	Control	Statroltea
WBC (10^9/L)	13.20 ± 1.08[a]	18.70 ± 2.90[b]	7.06 ± 0.14[c]	9.05 ± 0.05[d]
RBC (10^{12}/L)	8.42 ± 1.48[ab]	9.80 ± 0.40[a]	7.73 ± 0.26[b]	7.46 ± 0.17[b]
Hemoglobin (g/dL)	14.03 ± 2.49[ab]	17.90 ± 1.50[a]	14.83 ± 0.40[b]	13.85 ± 0.49[b]
Platelets (10^{11}/L)	7.89 ± 1.17[ab]	8.91 ± 0.66[a]	6.70 ± 0.41[b]	6.85 ± 1.14[b]
Hematocrit (%)	42.93 ± 6.18[a]	52.60 ± 2.51[b]	41.93 ± 2.65[a]	39.25 ± 1.77[a]
MCV (fL)	52.20 ± 0.71[a]	63.90 ± 1.70[b]	54.33 ± 2.71[a]	52.65 ± 1.20[a]
MCH (pg)	17.55 ± 0.35[a]	17.85 ± 0.49[ab]	19.20 ± 1.14[b]	18.50 ± 0.28[b]
MCHC (g/dL)	33.65 ± 0.21[a]	32.20 ± 0.18[b]	35.43 ± 2.76[ac]	35.25 ± 0.35[c]
RDW (%)	20.25 ± 0.35[a]	21.40 ± 0.90[a]	18.90 ± 1.87[a]	19.05 ± 1.67[a]
PCT(%)	0.54 ± 0.08[a]	0.69 ± 0.01[b]	0.52 ± 0.04a	0.48 ± 0.08[a]
MPV (fL)	7.33 ± 0.55[a]	8.20 ± 1.13[a]	7.83 ± 0.46[a]	6.95 ± 0.07[b]
PDW	14.90 ± 0.10[a]	15.05 ± 0.07[a]	15.20 ± 0.79[ab]	14.55 ± 0.21[b]

Values with different letters are significantly different (P < 0.05)

3.2. Effect of 60-days administration of Statroltea on the relative weight of vital organs

The relative weights of internal organs of Statroltea- treated are summarized in table 4. Although the weight of many organs (heart, lungs) has not been influenced after Statroltea consumption, some relevant changes have been noticed. They include significant decrease (P < 0.05) in relative liver and kidneys weight of both animals. Numerous authors have observed an abnormal increase of both liver and kidney weights in diabetic rats (Singh *et al.,* 2005; Vats *et al.,* 2003). Statroltea by producing a significant decrease in the relative weight of kidneys and liver of rats may have a positive impact on the health of animals.

Table 4: Effect of Statroltea on relative organ weights of male rats

Organs (mg/g BW)	Standard diet		High-fat diet	
	Control	Statroltea	Control	Statroltea
Heart	3.38 ± 0.22[a]	4.39 ± 1.57[a]	3.59 ± 0.03[a]	3.45 ± 0.64[a]
Liver	34.32 ± 1.66[a]	27.31 ± 3.37[b]	40.12 ± 2.22[c]	32.22 ± 3.40[a]
Lungs	9.31 ± 2.21[ab]	7.27 ± 2.10[a]	13.83 ± 2.79[b]	10.39 ± 3.43[b]
Kidneys	8.88 ± 2.29[ab]	6.11 ± 0.95[a]	8.95 ± 0.45[b]	6.67 ± 0.46[a]
Brain	6.19 ± 0.78[a]	8.55 ± 0.21[b]	5.51 ± 0.28[a]	5.65 ± 0.14[a]
Spleen	3.04 ± 0.41[a]	2.73 ± 0.50[a]	3.73 ± 0.10[a]	2.67 ± 0.15[a]

Values with different letters are significantly different (P < 0.05)

3.3. Effect of 60-days administration of Statroltea on liver function

Liver damage may consist of hepatocellular necrosis, cholestasis or a mixture of biochemical and histopathological patterns. The determination of ALT, AST and ALP or all three is useful in the early diagnosis of viral or toxic hepatitis and therefore, in studying patients exposed to hepatotoxicity. ALT is a hepatospecific enzyme that is principally found in the cytoplasm of rats. AST is an enzyme that is present in high quantities in the cytoplasm and mitochondria of liver, also present in the heart, skeletal muscle, kidney and brain. It is known that increase in the enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture. After severe damages, AST levels rise 10 to 20 times and greater than normal, whereas ALT can reach higher levels (up to 50 times greater than normal). On the other hand, a rise in serum ALP level is usually a characteristic finding in cholestatic liver disease (Huang *et al.,* 2006; Vozarova *et al.,* 2002).

The effect of Statroltea on liver function is displayed in table 5.

Table 5: Effect of Statroltea on liver function indexes of male rats

Parameter	Standard diet		High-fat diet	
	Control	Statroltea	Control	Statroltea
AST (UI/L)	85.10 ± 7.52[a]	90.35 ± 6.72[a]	123.25 ± 3.96[b]	128.73 ± 9.65[b]
ALT (UI/L)	19.78 ± 2.55[a]	20.48 ± 1.48[a]	25.32 ± 3.46[a]	22.66 ± 3.17[a]
ALP (UI/L)	44.85 ± 4.89[a]	58.63 ± 5.46[b]	31.74 ± 2.78[c]	91.08 ± 0.50[d]
Glucose (mg/dL)	60.50 ± 0.71[a]	77.00 ± 0.50[b]	65.33 ± 9.07[ab]	90.50 ± 9.19[c]
Total protein (g/dL)	5.97 ± 1.02[a]	6.60 ± 1.12[a]	5.93 ± 0.95[a]	6.25 ± 0.81[a]
Albumin (g/dL)	3.37 ± 0.82[a]	2.75 ± 0.33[a]	2.75 ± 0.36[a]	3.92 ± 0.60[a]

Values with different letters are significantly different ($P < 0.05$)

This table shows that ingestion of Statroltea caused no significant change in ALT and AST activities in male rats during the study and all values are within the normal ranges. On the other hand, ALP activity has been significantly increased in rats after consumption of Statroltea. However, it is worth mentioning that this ALP activity remains within the normal range (30-90 UI/L).

Table 5 also displays an increase in the blood glucose provoked by Statroltea ingestion irrespective of diet. However, the blood glucose level of Statroltea-treated rats is found to be within the normal range (70-119 mg/dL). This augmentation is controversial to the finding of Renno *et al.* (2008) who reported that the level of serum glucose was lower in the green tea-treated rats. This controversy may be explained by the high reducing sugars content of Statroltea compared to green tea.

The determination of serum proteins can act as a criterion for assessing synthetic capacity of the liver since nearly all of them are synthesized in hepatocytes. A decrease in plasma proteins therefore tends to reflect chronic damage. Albumin is the most abundant circulatory protein and its synthesis is a typical function of normal liver cells. The common pattern seen following significant hepatocellular damage is a reduction in albumin accompanied by a relative increase in globulins which leads to albumin/globulin ratio reduction, often with changes in the level of total protein (Rasekh *et al.,* 2008). Table 5 reveals that albumin and total protein levels are not affected by Statroltea in both experiments, confirming that Statroltea caused no damage in hepatic cells. In the same light, Liu *et al.* (2003) reported that tea polyphenols modified neither total protein and nor albumin levels when administrated intra-gastrically at doses of 833 mg/Kg for six months to standard-diet fed male and female rats.

3.4. Effect of 60-days administration of Statroltea on renal function

Kidney functions were evaluated through the concentration of uric acid, creatinine, and electrolytes in blood. Uric acid and creatinine blood levels can be used as a rough index of the glomerular filtration rate. Increased blood creatinine is a good indicator of negative impact in kidney functions (Rhiouani *et al.,* 2008). The effect of Statroltea on renal function is summarized in table 6.

Table 6: Effect of Statroltea on renal function indices of male rats

Parameters	Standard diet		High-fat diet	
	Control	Statroltea	Control	Statroltea
Creatinine (mg/dL)	0.94 ± 0.17[a]	0.82 ± 0.05[a]	0.99 ± 0.19[a]	1.08 ± 0.08[a]
Uric acid (mg/dL)	4.92 ± 0.42[a]	3.67 ± 0.52[bc]	4.08 ± 0.30[b]	3.13 ± 0.18[c]
Na^+ (µg/ml)	187.50 ± 4.75[a]	209.87 ± 2.70[b]	375.00 ± 3.25[c]	437.5 ± 5.16[d]
K^+ (µg/ml)	20.83 ± 1.40[a]	18.22 ± 2.56[ab]	13.02 ± 3.01[b]	18.23 ± 3.68[ab]
Fe^{2+}(µg/ml)	2.40 ± 0.52[a]	1.89 ± 0.13[a]	1.83 ± 0.08[a]	1.83 ± 0.01[a]

Values with different letters are significantly different ($P < 0.05$)

Table 6 shows that serum concentration of creatinine is not changed in Statroltea-treated rats irrespective of diet, revealing the kidney protective effect of Statroltea. This is concomitant with Chengelsis *et al.* (2008) who reported that heat-sterilized green tea catechins administered for 28 days on female or male rats at 2000 mg/kg/day produced no significant change in serum creatinine level of these animals. Table 6 also exhibits the effect of Statroltea on the uric acid level. Hyperuricemia, characterized by high serum uric acid level has been considered an important risk factor for gout and may be associated with development of such disorders as cardiovascular diseases, hyperglycemia/diabetes mellitus, alcoholism, renal failure, obesity, dyslipidemia, AIDS, cancer and increased mortality (Choi *et al*., 2004). Statroltea after consumption produced a decrease in the uric acid level in serum showing that Statroltea can be used to reduce metabolic diseases.

A decrease in serum sodium is found frequently in liver cirrhosis. The disruption of Na-K-ATPase function causes sodium flow to the cell on the basis of concentration gradient which leads to distributional hyponatremia. Hyponatremia also occurs due to decreased plasma volume during treatment with diuretics. Meanwhile hypokalemia is a common complication in liver cirrhosis, hyperkalemia may occur in renal failure (Jarcuska *et al*., 2004). Table 6 presents a significant increase in sodium level and constancy in the potassium concentration after consumption of Statroltea by rats ($P < 0.05$). The enhancement of sodium level in the sera reflects the protective function of Statroltea since promotion of sodium excretion is a sign of kidney failure. According to Nwafor *et al*. (2004), potassium is the principal intracellular cation and because its level has not changed in Statroltea- treated rats compared to control rats; it can be assumed that the integrity of the cells has not been compromised. In the same light, Chengelsis *et al*. (2008) reported that serum level of potassium was not influenced by that heat- sterilized green tea catechins administrated for 28 days to female or male rats up to 2000 mg/kg/day.

On the other hand, the level of blood iron was not influenced by Statroltea ingestion irrespective of diet. This is controversial to tea from *C. sinensis* which was reported to be a potent inhibitor of iron absorption by forming insoluble complexes with iron within the gastrointestinal tract (McKay and Blumberg, 2002).

Conclusions

The hematological parameters, the relative organ weights and biochemical functions have been assessed to evaluate the safety of Statroltea. The hematogical analyses reveal that the consumption of Statroltea increases WBC count in male rats and has a good potential on immune function. The ingestion of Statroltea produces a significant decrease in the relative weight of kidneys and liver of rats and may have a positive impact on the health of these animals. The administration of Statroltea causes no damage in hepatic cells revealed by stagnation in ALT activity and serum proteins level. On the other hand, Statroltea exercises a protective effect on kidneys through stagnation in the serum level of creatinine and a decrease in serum level of uric acid irrespective of diet.

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