The 1st International Conference on Local Resource Exploitation
www.lorexp.org / info@lorexp.org
REF: LOREXP_2021_A1096 Pages: 400–410

LOREXP
LOCAL RESOURCE EXPLOITATION

Effect of palm olein supplemented with old Cameroonian green leave tea extract on lipid profile and some hematological parameters on rat during frying.

Effet de l'oléine de palme additionnée d'un ancien extrait de thé vert camerounais sur le profil lipidique et certains paramètres hématologiques chez le rat lors de la friture

Loungaing Demgne Valerie[1,2], Fabrice Tonfack Djikeng[1], Gires Boungo Teboukeu[1] and Hilaire Macaire Womeni[1,*]

[1] Research Unit of Biochemestry, Medicinal Plants, Food Sciences, and Nutrition, department of Biochemestry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon.
[2] Institute of Agricultural Research for Development, Foumbot Multipurpose Station P.o. box: 163 Foumbot.
* Corresponding Author: womeni@yahoo.fr

RÉSUMÉ :

Le pouvoir antioxydant des extraits méthanoliques de vieilles feuilles de thé n'est plus à démontrer. Le présent travail vise à contribuer à la recommandation des vieilles feuilles de thé vert comme source d'antioxydant pour la stabilisation de l'oléine de palme. L'oléine de palme a été stabilisée à 1800 ppm d'extrait de feuilles de thé et 200 ppm de BHT, oléine de palme sans antioxydant représentait le contrôle négatif. Tous ces échantillons d'huile ont été soumis à 5 cycles de friture puis, employés pour les tests *in vivo*. 35 rats de souche wistar répartis en 7 groupes de 5 chacun ont été utilisés. Le premier groupe représentait le groupe témoin neutre et ne consommait que l'aliment standard. Les 6 autres groupes ont reçu les différents échantillons d'huile par supplémentation alimentaire à raison d'un échantillon par groupe. Il ressort de ces travaux que la consommation de l'oléine de palme contenant l'extrait méthanolique de vieilles feuilles de thé a conduit à une diminution significative ($p < 0,05$) du cholestérol total, du cholestérol LDL et des triglycérides, suivie d'une augmentation significative ($p < 0,05$) du cholestérol HDL. Ces extraits ont également contribué à restaurer les taux de globules rouges et d'hématocrite après la friture. Les extraits de vieilles feuilles de thé présents dans l'oléine de palme fraîche agissent comme un prooxydant en réduisant le taux de globules rouges, d'hématocrite et d'hémoglobine.

Mots clés : Vieilles feuilles de thé, Oléine de palme, Friture, Antioxydant, BHT, Rat wistar.

ABSTRACT:

The antioxidant power of old Cameroonian green leaves tea methanolic extracts is no longer to be demonstrated. The present work aims to contribute to the recommendation of the use of old tea leaves as an antioxidant source for the stabilization of palm olein. Palm olein was stabilized with 1800 ppm of old leave tea extract and 200 ppm of BHT, palm olein free antioxidant represented the negative control. All of these oil samples were subjected at 5 cycles of frying. The oil samples were tested both fresh and fried during the *in vivo* tests. 35 wistar rats divided into 7 groups of 5 each were used. The first group represented the neutral control group and consumed only the standard food. The other 6 groups received the various oil samples by dietary supplementation, one sample per group. It emerges from this work that the consumption of the palm olein containing old leave tea extract led to a significant decrease ($p < 0.05$) of total cholesterol, LDL cholesterol and triglycerides and a significant increase ($p < 0.05$) of HDL cholesterol. These extracts also contributed to restore red blood cell and hematocrit levels after frying. Old leave tea extracts present in fresh palm olein act such as prooxidant by reducing red blood cell, hematocrit and hemoglobin level.

Keywords: Old leave tea, Palm olein, Frying, Antioxidant, BHT, Wistar rat.

1. INTRODUCTION

Deep frying is a very old food cooking technique. It is highly regarded and widely used by people around the world. The implementation of this process involves the immersion of the food in oil brought to high temperature (160 - 180 °C), it is generally carried out in an open bath in the presence of air (Sukumar et al., 2012) and during a well-defined period. Under these conditions, the oil degrades through various reactions such as hydrolysis, oxidation, polymerization and isomerization (Kamsiah et al., 2011; Gupta et al., 2014). These reactions are responsible of the formation of free radicals and several other volatile and non-volatile compounds which, when ingested into the body through food, have the particularity of being toxic. Indeed according to Leong et al. (2008) and Leong et al. (2012), free radicals from oil degradation during heating process can cause elevation in blood pressure and damage membrane lipids through lipid peroxidation and thus induce oxidative stress. In addition, ketone compounds, alcohols and aldehydes which are produced during frying are cytotoxic and their ingestion through fried food can damage DNA and lead to cell necrosis (Cheng-Huang et al., 2006; Eshak et al., 2010). Another compounds such as trans fatty acids generally leads to an increase in LDL cholesterol and a decrease in HDL cholesterol (Brower et al., 2010) resulting in the development of cardiovascular disease.

In view of all this, the use of antioxidants remains the best option to limit the degradation of the oil and therefore the formation of toxic compounds during frying. Butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA) and ter-butylhydroquinone (TBHQ) are chemical additives generally used in the food industry to limit the oxidation of oils and fats. The problem with these synthetic compounds is that they are not completely effective because their capacity to protect the oil is reduced at high temperature, in fact during frying they tend to volatilize and to infiltrate in the food (Choe and Min 2007). Further according to Botterweck et al. (2000) their long-term use represents a health hazard because of their toxic effect. According to (Kahl and Kappus, 1993), these synthetics antioxidants are tumors and carcinogens promoters. In view of all this, several research works have been carried out with the aim of identifying plants with antioxidant characteristics.

In Cameroon, old green leave tea which are mature leaves are found in the base of tree, they are often eliminated when cleaning the farm. Following this observation, Womeni et al. (2016) have shown that the methanolic extracts of old Cameroonian green leave tea contain 53.5 mg EAG/g of phenolic compounds and that they have the capacity to limit the oxidation of palm olein in the same way as BHT when stored in an oven at 70 °C for 30 days. In the same launch, Tonfack et al. (2017) showed that the use of these extracts at the concentration of 200 - 1800 ppm also delays the oxidation of palm olein when subjected to 180 °C for 6 days, and the best stability of palm oil sample was observed when they used old Cameroonian green leave tea extracts at 1800 ppm.

The use of these extracts to date in the oxidative stability of palm olein has only been tested *in vitro*. It is therefore of great health interest to know whether these extracts can protect palm olein during frying to the point of limiting the deleterious effects of toxic products resulting from the degradation of palm olein in

the body. During deep fat frying, the toxic compounds that form are absorbed by the food, which also makes it harmful to the end consumer. With a view to contributing to the recommendation of old green tea leaves as an antioxidant source for the stabilization of palm olein the focus of this work was to study of the effect of palm olein stabilized with methanolic extracts from old green tea leaves on the lipid profile and some hematological parameters of rats.

2. MATERIALS AND METHOD

2.1. Material

The old leaves of *Camellia sinensis* were harvested from the Djuttitsa Tea Estate plantation in the Menoua department in western Cameroon were an altitude is 1.850, average annual rainfall are 1717.7mm and average temperature is about 19.7 °C. The palm olein without additives was purchased from SCS/RAFCA, a refinery company based in Bafoussam also in the West Cameroon region. All the chemicals and reagents used were of analytical reagent grade.

2.2. Method

2.2.1. Extraction of tea leave antioxidant

This was done according to the modified method of Tonfack et al. (2017). The old leave tea freshly collected were cleaned and dried in an oven at 50 °C for 48 hours. They were crushed and then sieved using a 1 mm diameter sieve. 250 g of powder were macerated in 1 L of methanol at room temperature with regular stirring for 48 h. Wattman No. 1 paper was used to filter the extracts. The filtrate obtained was placed in an oven at 45 °C for 48 h in order to remove the solvent until the extract became solid and the weight became constant. The resulting extract was stored in a refrigerator at 4 °C for stabilization of palm olein.

2.2.2. Stabilization of palm olein with tea leave extract and BHT

The operation was carried out according to the modified method of Tonfack et al. (2017). The concentrated plant extract was diluted in 5 ml of methanol and added to 1.5 kg of palm olein (previously preheated to 50 °C for three hours) at concentrations of 1800 ppm. BHT was used at its recommended concentration which is 200 ppm. The palm olein free antioxidant was prepared under the same conditions as described above. The samples thus formed were shaked regularly for 30 min and were placed in an oven at 45 °C for 48 h in order to reduce the amount of methanol present in the medium. Extract was used at 1800 ppm, because Tonfack et al. (2017) have found the best stability of palm olein when they used *Camellia sinensis* at this concentration.

2.2.3. Frying palm olein

Before deep frying, 100 ml of each sample of oils previously formed was collected. The modified protocol of Leong et al. (2015) was used for this step. The palm olein sample was heated for 5 minutes at 180 °C in an electric fryer, then 50 g of unripe plantain previously cleaned and cut into strips was introduced. After 3

minutes, the plantain chips were removed from the oil. The whole 8 minutes thus represented one time frying. The operation was repeated 4 times to obtain 5 time frying oil. Then the sample oil was cooled to room temperature for 5 hours. All oil samples (stabilized and unstabilized) were prepared under the same conditions and there were a total of 6 oil samples to be used for in *vivo test*.

2.2.4. Preparation of diet and treatment of animals

Thirty-five (35) wistar rats weighing 150 - 180 g were individually divided into 7 groups of 5 animals each acclimatized during one week before the experience. The supplementation of the oil sample to be tested in the animal diet was done daily, for this purpose the supplementation of oil in diet was done at 20 ml/kg of diet. Each animal received 25 g of diet supplemented with test oil. Table 1 shows the composition of animal's diet.

Table 1: Composition of animal's diet

Ingredients	Ingredient for 100 g
Corn flour	68 g
Soy flour	20 g
Fish meal	10 g
Borne meal	1 g
Cooking salt	0,8 g
Tested oil	2 ml
Vitamin and mineral mix	0,1g

All had free access to water and was subjected to 12 hours light/dark cycle, the temperature of the study environment was 20.9 ° C. The test was carried out of a period of 30 days. The different groups were made up as follows:

Group 1, which served as a neutral control, consumed only the standard food.

Groups 2 and group 3 (PO+BHT and FPO+BHT) were positive controls and received daily by dietary supplementation fresh and frying palm olein stabilized with BHT respectively.

Groups 4 and group 5 (PO and FPO) were considered as negative controls and received daily by food supplementation unstabilized fresh and frying palm olein respectively.

Groups 6 and group 7 (PO+CaS1800 and FPO+CaS1800) received daily by food supplementation fresh and frying palm olein stabilized at 1800 ppm of extract respectively.

All experiments were carried out according to the regulations and ethical approval of the Experimental Animal Welfare and Ethic Committee of the institution.

2.2.5. Evaluation of weight gain by animals during the consumption of different oil samples

The weight gain was evaluated every day and the percentage was determined as follows:

$$P\% = \frac{wf - w0}{wf} * 100$$

P% = percentage of weight gain on day x

W0 = body weight of animal on day x

Wf = initial body weight of animal (after the beginning of experience)

2.2.6. Sample collection

At the end of the experiment, the animals were anesthetized with chloroform vapor, then dissected and blood was collected by cardiac puncture using a syringe. The blood of each animal was introduced into two different tubes (tubes containing disodium ethylene diaminetetracetic acid (EDTA) for hematological tests and dry tubes for serum separation). The serum was obtained by centrifugation of the blood collected in the dry tubes at 3000 rpm for 15 min. The supernatant was removed using aliquots and introduced in epindorfs. The whole was stored at -30 °C for subsequent biochemical tests.

2.2.7. Determination of lipid profile

Enzymatic kits (SPINCTREACT) were used to determine triglyceride (TG), total cholesterol (TC) and high density lipoprotein (HDL).

And low-density lipoprotein (LDL) were calculated by Fridewalds formula as given below and the atherogenic indexes (AI) were calculated as well.

LDL= TC - (HDL - (TG/5)) and AI = TC/HDL

2.2.8. Evaluation of hematological parameters

The hematological analysis was performed by using an automated blood cells counter (SFRI H18 LIGHT) with standard calibration.

2.2.9. Statistical analyzes

SPSS software version 23.0 for Windows was used for statistical analyzes. All values were expressed as mean ± standard deviation. The results were analyzed by analysis of variance (ANOVA). The Waller-Duncan test was used for the difference in means between the different groups. The test was considered to be significantly different when $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of consumption of different sample oils on weight gain of animals

Figure 1 shows the effect of consumption of different fresh and frying oil on weight gain of animals during the experiment. All animals has shown a significant increase of weight gain during the test. The highest weigh gain is observed in group PO+BHT and group FPO+BHT which are 36.61 % and 36.09 %

respectively and the lowest weigh gain is observed in neutral control group (29.95 %). This increase of weight gain observed in groups tests compare to neutral group would be due to the consumption of oil present in the diet. In fact, consumption of excess fat in the diet leads to a reserve of energy in adipose tissue in the form of triglycerides and thus promotes weight gain. These results are in accordance with those obtained by Narasimhamurthy and Raina (1999); Siti et al., (2008). Who shown that consumption of fresh and frying oil increase the body weight of animal compare to the control which not consume oil. In addition, it also emerges from the work of Leong et al. (2010) that the consumption of food supplemented with fresh or fried soybean oil leads to an increase in the body mass of the animals.

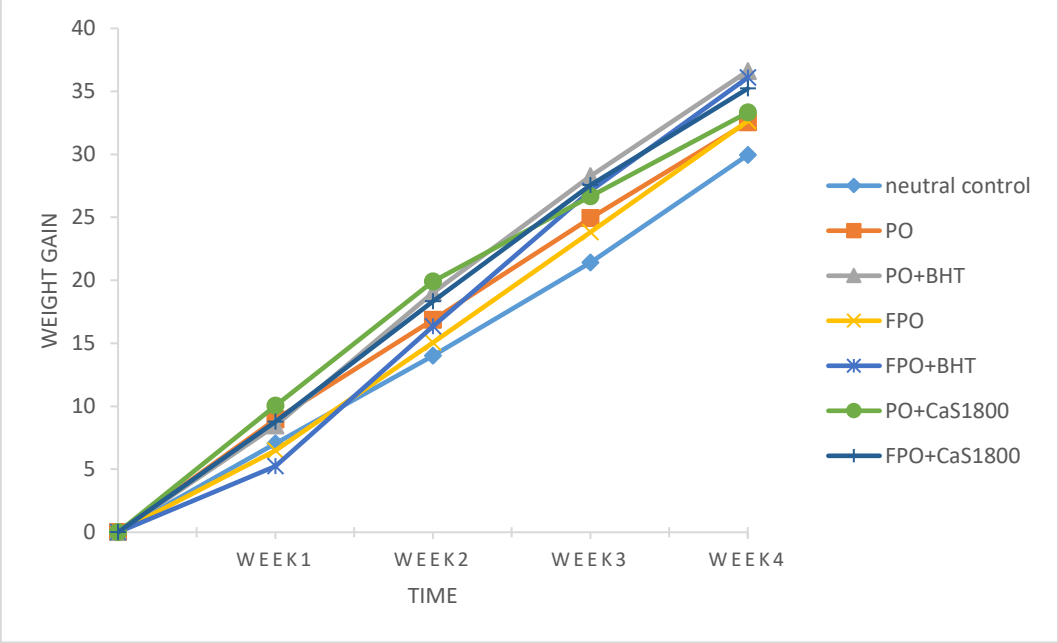

Figure 1: Evolution of weight gain of animals during experiment.

Neutral control received standard diet; PO received standard diet with unstabilized palm olein; PO+BHT received standard diet with palm olein containing BHT; PO+CaS1800 received standard diet with palm olein containing 1800 ppm of old leave tea extracts; FPO received standard diet with unstabilized frying palm olein, FPO+BHT received standard diet with frying palm olein containing BHT and FPO+CaS1800 received standard diet with frying palm olein containing 1800 ppm of old leave tea extracts.

3.2. Effect of consumption of different sample oils on lipid profile of animals

Table 2 shows the effect of the consumption of different palm olein samples on the lipid profile of the animals. It appears that the consumption of palm olein enriched with methanolic extract of old tea leave resulte in a significant decrease ($p < 0.05$) in triglycerides. The PO+CaS1800 and FPO+CaS1800 groups exhibited serum triglyceride concentrations of 133.29 mg/dL and 138.02 mg/dL respectively. Now the PO+BHT, FPO+BHT, PO and FPO groups have respective concentrations of 144.78 mg/dL; 142.30 mg/dL; 167.09 mg/dL and 183.09 mg/dL. Moreover, the PO+CaS1800 and FPO+CaS1800 groups do not show any significant difference ($P>0.05$) with regard to the serum concentrations of total cholesterol, LDL cholesterol and atherogenicity index in comparison with the PO+BHT groups and FPO+BHT. The presence of old tea

leave extract in palm olein contributed also to the significant increase in HDL cholesterol compared to consuming palm olein without additives. No significant difference was observed in the PO+BHT, FPO+BHT groups compared to the PO+BHT group and the neutral control.

Table 2 Effect of different sample palm olein on the lipid profile of animals

	Treatments						
Parameters	Neutral control	PO+BHT	FPO+BHT	PO	FPO	PO+CaS1800	FPO+CaS1800
TAG (mg/dL)	$85.63 \pm 3.30^{a}{}_{A}$	144.78 ± 2.73^{d}	142.30 ± 2.66^{cd}	167.09 ± 3.12^{e}	183.09 ± 2.67^{f}	133.29 ± 2.83^{b}	138.02 ± 3.00^{c}
TC (mg/dL)	$69.61 \pm 1.85^{a}{}_{A}$	91.20 ± 3.74^{b}	97.25 ± 1.87^{d}	103.82 ± 2.90^{e}	109.20 ± 1.13^{f}	91.95 ± 2.63^{bc}	96.43 ± 4.22^{cd}
HDL (mg/dL)	67.82 ± 4.06^{cd}	66.63 ± 6.47^{cd}	62.15 ± 2.88^{bc}	59.58 ± 2.03^{b}	50.25 ± 5.20^{a}	71.94 ± 1.49^{d}	69.93 ± 1.84^{d}
LDL (mg/dL)	$18.91 \pm 5.57^{a}{}_{A}$	53.52 ± 8.90^{b}	63.56 ± 3.69^{c}	77.65 ± 3.14^{d}	95.57 ± 4.95^{e}	46.66 ± 2.32^{b}	54.10 ± 5.51^{b}
AI	$1.02 \pm 0.07^{a}{}_{A}$	1.38 ± 0.16^{bc}	1.56 ± 0.08^{cd}	1.74 ± 0.06^{d}	2.19 ± 0.22^{e}	1.27 ± 0.02^{b}	1.38 ± 0.08^{bc}

The values of this table represent the means ± standard deviation of 5 repetitions, (a-f) Means within each row for the same parameter with different superscripts are significantly ($p < 0.05$) different. Neutral control received standard diet; PO received standard diet with unstabilized palm olein; PO+BHT received standard diet with palm olein containing BHT; PO+CaS1800 received standard diet with palm olein containing 1800 ppm of old leave tea extracts; FPO received standard diet with unstabilized frying palm olein, FPO+BHT received standard diet with frying palm olein containing BHT and FPO+CaS1800 received standard diet with frying palm olein containing 1800 ppm of old leave tea extracts. TAG=Triglycerides, T-CHOL=Total Cholesterol; HDL=High Lipoprotein; LDL=Low Density Lipoprotein; AI= Atherogenic Index

According to Chun-Yi et al. (2014) long-term consumption of reheated oil several times results in an increase in blood pressure and total cholesterol which predisposes to atherosclerosis. This attests to the observation made in the context of this work. The FPO group exhibited significantly elevated concentrations of triglycerides, total cholesterol and LDL cholesterol which were respectively 183.09 mg/dL, 109.20 mg/dL and 95.57 mg/dL followed by the lowest concentration of HDL cholesterol 50.25 mg/dL. These results reflect the risk of cardiovascular disease. This could be explained by the presence of free radicals formed in palm olein during frying. In addition, when an oil is subjected thermal treatment, several reaction mechanisms contribute to its degradation, including isomerization reactions that result in the formation of trans fatty acids (Cuvelier and Maillard, 2012). Consumption of these fatty acids leads to an increase in LDL cholesterol and a reduction in HDL cholesterol (Khor and Mohd, 2008; Soelaiman and Jaarin, 2008; Ayodeji et al., 2017). These results are in agreement with those of Badr El Said et al. (2015) and Garrido-Polonio et al. (2004) who found that administration of thermoxidized vegetable oils to rats resulted in an increase in triglycerides, total cholesterol and LDL cholesterol followed by a reduction in HDL cholesterol. Similarly, it emerges from the work of Adam et al. (2008) that the consumption of heated palm oil causes an increase in total cholesterol in postmenopausal rats. The PO+Cas1800 and FPO+CaS1800 groups presented a better lipid profile with a significant increase ($P < 0.05$) in HDL

cholesterol, namely 71.94 mg/dL and 69.93 mg/dL respectively. This would be due to the presence of natural antioxidants present in the methanolic extracts of old leaves tea. These would have given good oxidative stability to palm olein before and after frying. It emerges from the work of (Womeni et al., 2016) that the methanolic extracts of old leaves tea are rich in gallic acid, epicatechin gallate, gallocatechin and epigallocatechin gallate which are very powerful antioxidants, the latter would have acted by giving their proton to stabilize free radicals formed in the oil during treatments. Similarly, Tonfack et al. (2017) revealed that the presence of these extracts in palm olein limits the conversion of linoleic acid to trans linoleic acid during storage at 180 °C for 6 days at a rate of 3 hours of heating per day. These results agree with those of Shila et al. (2011) who showed that consumption of oxidized oil supplemented with pectin fiber improves the lipid profile of rats. They are also in agreement with those of Kurtoglu et al. (2008) who found that dietary supplementation of 0.05 % vitamin E improves the lipid profile of rats when they consume olive oil.

3.3. Effect of oil sample on some hematological parameters

Table 3 shows the effect of consuming different oil samples on RBC, WBC, HGB, HCT, MCV and MCHC. It appears that no significant difference ($p > 0.05$) is observed for WBC, MCV and MCHC between all the groups. However, the PO + CaS1800 group has shown the lowest concentration of RBC, HGB and HCT which is 6.79 10^6/µL; 13.72 g/dL and 42.28 % respectively.

The FPO + CaS1800 group exhibits concentrations of RBC, HGB and HCT which are 8.08 10^6/µL; 16.52 g/dL and 50.90 % respectively. These concentrations are similar to those observed in the neutral control group. The low concentration of RBC, HGB and HCT observed in the PO+CaS1800 group could be the consequence of the prooxidant effect caused by the phenolic components presents in the old leave tea extracts in the oil. When the antioxidant concentration is high in an organism, the latter behave like prooxidants by producing free radicals (Eghbaliferiz and Iranshahi 2016).

These free radicals would have reacted by suppressing the process of growth and differentiation born marrow. Moreover, free radicals would have reduced RBCs concentration by increasing their membrane fragility then, the decrease of RBCs lead the decrease of HCT. The decrease of HGB can be attributed to loss of iron by the liver due to destruction of liver tissues by free radicals (Mesembe et al., 2004). Inversely, The increase in these parameters observed in the FPO+CaS1800 group would be due to the antioxidant effect of the extracts of old leave tea because the frying would have allowed a partial loss of these extracts by volatilization and therefore the phenolic components present in this extract currently act by giving a proton to free radical formed during treatment allow to protect the palm olein from oxidation during frying. According to Womeni et al. (2016) methanolic extract of old Cameroonian green leave tea are the powerful free radical scavenger and a powerful ferric reducer. These results are in disagree with those of Mesembe et al. (2004) and Hussein, (2015) who has found that the consumption reheated oil cause decrease of RBC, HGB and HCT cells when compare to the fresh palm oil and control group. This disagree would be due to

the presence of extract in oil, the difference in the number of cycles frying, type of oil and treatment duration.

Table 3: Effect of oil sample on some hematological parameters.

Parameters	Treatments						
	Neutral control	PO+BHT	FPO+BHT	PO	FPO	PO+CaS1800	FPO+CaS1800
RBC (10^6/µL)	8.96 ± 0.37^{b}	7.29 ± 0.85^{ab}	7.42 ± 0.96^{ab}	7.50 ± 0.83^{ab}	7.33 ± 1.00^{ab}	6.79 ± 1.78^{a}	8.08 ± 0.93^{ab}
WBC (/10^3/µL)	3.04 ± 1.11^{a}	4.26 ± 1.86^{a}	3.90 ± 1.45^{a}	4.26 ± 2.01^{a}	5.24 ± 1.89^{a}	5.46 ± 3.40^{a}	5.28 ± 2.82^{a}
HGB (g/dL)	16.02 ± 0.76^{ab}	15.46 ± 0.87^{ab}	15.64 ± 1.23^{ab}	15.52 ± 0.59^{ab}	15.40 ± 1.61^{ab}	13.72 ± 2.87^{a}	16.52 ± 1.15^{b}
HCT (%)	52.48 ± 1.75^{b}	46.22 ± 4.46^{ab}	45.42 ± 4.58^{ab}	46.94 ± 2.09^{ab}	45.84 ± 2.03^{ab}	42.28 ± 9.53^{a}	50.90 ± 5.31^{b}
MCV (fl)	62.86 ± 4.02^{a}	63.08 ± 2.89^{a}	60.92 ± 2.48^{a}	61.92 ± 1.22^{a}	64.38 ± 2.84^{a}	63.62 ± 3.92^{a}	61.22 ± 2.84^{a}
MCHC (g/dL)	34.26 ± 3.28^{a}	33.84 ± 2.55^{a}	33.20 ± 0.89^{a}	34.18 ± 1.72^{a}	34.46 ± 1.31^{a}	30.66 ± 2.03^{a}	33.30 ± 2.61^{a}

The values of this table represent the means ± standard deviation of 5 repetitions, (a-b) Means within each row for the same parameter with different superscripts are significantly ($p<0.05$) different Neutral control received standard diet; PO received standard diet with unstabilized palm olein; PO+BHT received standard diet with palm olein containing BHT; PO+CaS1800 received standard diet with palm olein containing 1800 ppm of old leave tea extracts; FPO received standard diet with unstabilized frying palm olein, FPO+BHT received standard diet with frying palm olein containing BHT and FPO+CaS1800 received standard diet with frying palm olein containing 1800 ppm of old leave tea extracts.. RBC: red blood cell count; WBC: white blood cell count; HGB: hemoglobin; HCT: hematocrite; MCV: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

4. CONCLUSION

This work focused on the effect of palm olein supplemented with extracts of old Cameroonian green leave tea on lipid profile and some hematological parameters in rats. As a result, the presence of methanolic extracts of the leaves of old Cameroonian green leave tea improve lipid profile of animals. The use of these extracts at 1800 ppm shows better efficacy than BHT on the protection of HDL cholesterol and after 5 time frying the presence of the extract in palm olein during frying also contributed to increase RBC, HCT and HGB. These extracts can be recommended in the food industry in order to stabilize palm olein.

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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