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Effect of *Mbuja* Oil Consumption Compare to Palm Olein and Corn Oils on Renal and Liver Dysfunction Factors in Wistar Rats

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Abstract: *Mbuja* (*Bikalga; dawadawa botso; datou; Furundu*) is a food condiment obtained by a traditional uncontrolled fermentation of *Hibiscus sabdariffa* seeds in African countries (Burkina Faso, Mali, Niger, Nigeria, Cameroon and Sudan). This condiment is known for its nutritive values and for its health properties. In spite of its nutritional and healthy properties the consumption of *mbuja* is less appreciated in urban areas. This is due to its strong smell, to its bad condition of manufacturing practices which leads to the rapid alteration of nutritive values. The main problem now is how to lead people to consume *mbuja* which nevertheless contains bioactive molecules, which can help in the treatment or in the prevention of some chronic diseases. In order to the valorisation of its nutraceutic property, a study on effect of *mbuja* oil consumption compare to palm olein and corn oils on renal and liver dysfunction factors in Rats was carried out. To overcome this, *mbuja* was purchased in Mokolo market (Far-North, Cameroon); oil extracted from it and nutraceutic aspect of oil was conducted after feeding male rats with different diets containing *mbuja*, corn and palm oils for 50 days. Renal and liver dysfunction factors was assessed by using classical methods. The results revealed that consumption of *mbuja* oil produces same effect as corn oil on renal and better effect on hepatic dysfunction factors. Consumption of *mbuja* oil improve kidney, heart and liver function. This confirms the hepatoprotective effect of *mbuja* and justify its use in traditional medicine to treat chronic diseases.

Keywords: *Mbuja*, Oil, Nutraceutic Activities, Dysfunction Factors, Hepatoprotective Effect

1. Introduction

Liver and kidney are the most vital organs in the human body which is involved in the regulation of various biochemical functions [1]. Damage of these organs occur due to inflammatory responses which are initiated by Kupffer cells activation releasing pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-a). They stimulate other cells to attract and activate circulating inflammatory cells [2]. To avoid damage of these organs, many studies suggest that the consumption of fruits and vegetables, rich in natural antioxidants, reduce the risk of chronic diseases [3]. In this context, plants rich in natural antioxidants, like phenolic compounds, have free radical scavenging ability with enhancement of the endogenous antioxidant enzymes (superoxide dismutase: SOD, catalase: CAT) as well as non-

enzymatic antioxidants (reduced glutathione: GSH) [1, 4]. Therefore, food plants rich with antioxidants could be potent renal and hepatoprotective agents [5]. Among african leguminous foods, that could have protective agents, *Mbuja* (*Bikalga; dawadawa botso; datou; Furundu*) is one of the traditional food condiment which have many therapeutic potentials. *Mbuja* is obtained by a traditional uncontrolled fermentation of *Hibiscus sabdariffa* seeds. This condiment is considered as basic food in many African countries (Burkina Faso, Mali Niger, Nigeria, Cameroon and Sudan among others) and it is known for its nutritive values (source of carbohydrates, dietary proteins among other nutrients) and for its health properties (contains bioactives compounds) [6, 7]. In Cameroon, *Mbuja* is mainly produced by women and constitute an economical source for the producers. This condiment is the most popular food condiments in Mokolo (Far-North, Cameroon); it is used as meat replacement mainly by low-income population, and also used in traditional medicine to cure high blood pressure and cardiovascular diseases or is used as an antiseptic, antioxidant and anti-inflammatory [6, 7]. In spite of its nutritional and healthy properties, consumption of *mbuja* is less appreciated in urban areas. This is due to its strong smell, to its bad condition of manufacturing practices which leads to the rapid alteration of nutritive values. The main problem now is how to lead people to consume *mbuja* which nevertheless contains bioactive molecules, which can help in the treatment or in the prevention of some cardiovascular diseases and inflammatory [7]. Previous studies on *mbuja* oil, revealed that its oil contained high crude phenolic contents, polyunsaturated fatty acids and potent *in vivo* antioxidant and hypocholesterolemic activity when compared with corn and palm oils [6, 7]. No scientific study on renal and liver protective effects of *mbuja* oil in the treatment of some renal and liver chronic diseases was found in literature review. Therefore, the goal of this study target nutraceutic valorization of *mbuja*. The present study was undertaken essentially to investigate the *in vivo* potential effects of *mbuja* oil on renal and liver dysfunction factors compared to corn and palm oils was assessed in the aim of the treatment of inflammatory and chronic hepatic diseases.

2. Material and Methods

2.1. Oil Sampling and Proximate Composition

The *mbuja* was purchased from various sellers from the Mokolo market in Far-North (Cameroon). The lipid composition was determined by exhaustively extracting a known weight of sample with hexane using a Soxhlet apparatus [8]. Corn and Palm olein oils were obtained commercially.

2.2. Animals and Diets

7 month old weaned male albinos Sprague Dawley rats (Harlan, France) weighing 260 ± 20 g were housed in polycarbonate cages in a controlled environment with a temperature of 25 ± 2°C, relative humidity (40–60%), with a 12-h light–dark cycle (12h/12h: 7 – 19 h light and 19 – 7h dark) [9]. During an acclimatization period of 1 week, the rats received tap water and a commercial rat diet ad libitu [10]. At the end of this period, the rats were weighed and randomly assigned to one of the three groups (n = 6 / group) according to diet composition. For 50 days, each group was fed a diet containing one of the following: *Mbuja* oil (MO group), corn oil (Lesieur France) (CO group) and palm olein oil (Palm'Or, Maya, Douala-Cameroun) (PO). Oil represented 5% of the composition of the diet as prescribed by American Institute of Nutrition [11]. The diet was reconstituted by using an alipidic diet (moisture content 8.53%, proteins 21.48%, dextrose 32.00%, starch 26.42%, cellulose 6.35%, mineral mix 4.58%, vitamins mix 0.64%). Animals had free access to water and food. Food was given each week and water twice per week.

2.3. Experimental Procedure

At the end of the feeding period (50 days), the rats fasted overnight (12 hours), then were weighed (to evaluate Body gain), anaesthetized under chloroform vapor and sacrificed. Blood samples were immediately collected from the heart by cardiac puncture in two tubes to obtain serum and plasma (heparin tubes). Serum was separated by centrifugation at 3000 rpm for 5 min (4°C) and plasma was separated by centrifugation at 1500 rpm for 10 min (4°C). Serum was used for creatinine, protein, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and bilirubin assessment.

After sacrificing the animals and collecting the blood, organs (liver, heart, kidneys,) were removed and weighed using a 1/1000 precision balance, Sartorius LP 620P, (AG Göttingen, Germany). The hepatosomatic (HSI) and viscerosomatic (VSI) indices were calculated as the percentage of liver or organ mass to the whole animal mass, respectively [12].

Investigation of renal function was done by assessment of serum creatinine and protein.

Serum creatinine is the most widely used method of assessing renal function. Serum creatinine is correlated with glomerular filtration rate [13]. The determination of creatinine in rat serum was carried out by the Jaffé method, with the Human Su-Crea 10052 kit. Total serum protein was done by Gornall *et al*. [14] method using the Human SU-PROT 10570 kit.

Liver function is determined by measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin. Determination of ALT and AST levels are based on Bergmeyer *et al*. [15] method by using Humans kits (EN-GPTU 12212 and EN-GTU 12211 for ALAT and ASAT respectively). Total bilirubin was evaluated following Winkelman *et al*. [16] method by using the Human SU-BILDT 10740 kit. The Mindray BA-88 analysers (Biochemistry Analyser, Manshan chenzhen 518057 P. R, China) coupled to the microprocessor automatically calculate the creatinine, protein, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and bilirubin concentration of each sample.

2.4. Statistical Analysis

Two samples are taken from the serum of the same rat. The result for each group is the average for 6 rats. The results obtained are expressed as the mean ± standard deviation. An analysis of variance, followed by a Duncan's multiple comparison test is used to determine the difference between the means of the treatments. Multiple correlations are used to investigate relationships between different factors. Statgraphics 5.0 [17] is used for these analyses. Effects at the $p<0.05$ probability level are considered significant. Experimental design is a randomised complete block.

3. Results and Discussion

3.1. Assessment of Somatic, Hepatosomatic and Viscerosomatic Parameters of Different Diets

Somatic parameters are parameters related to body weight gain, Hepatosomatic (liver mass/body mass) and viscerosomatic (organ/body mass) indexes of the rats are calculated to determine whether if different oils have an impact on organ function.

No mortality was recorded during the 50-day experimental period. Table 1 shows the hepatosomatic and viscerosomatic index of the rats based on the different diets.

Table 1. *Somatic, Hepatosomatic and viscerosomatic indexes of different diets.*

Parameters Groups	Initial Mass (g)	Final Mass (g)	Body mass Gain (%)	Heart Mass/Body mass (%)	Left kidney Mass/Body mass (%)	Right Kidney Mass/Body mass (%)	HSI (Liver mass /Body mass (%)
T0	255.47 ^{ab} (14.51)	288.79 ^{ab} (7.42)	12.84 ^{b} (1.16)	0.34 ^{a} (0.05)	0.37 ^{a} (0.6)	0.42 ^{a} (0.05)	3.72 ^{a} (0.6)
MO	271.26 ^{b} (21.24)	293.08 ^{ab} (2.56)	8.41 ^{a} (2.21)	0.36 ^{a} (0.02)	0.45 ^{a} (0.04)	0.44 ^{a} (0.04)	4.06 ^{ab} (0.31)
CO	237.17 ^{a} (12.70)	267.29 ^{a} (3.47)	12.69 ^{b} (1.07)	0.38 ^{a} (0.07)	0.43 ^{a} (0.4)	0.42 ^{a} (0.04)	4.44 ^{b} (0.54)
PO	265.54 ^{ab} (13.60)	298.86 ^{b} (5.91)	16.34 ^{c} (1.54)	0.36 ^{a} (0.01)	0.39 ^{a} (0.02)	0.42 ^{a} (0.2)	3.75 ^{ab} (0.27)

All results are means (Standard deviation) of six replicate experiments; values in the same column followed by the same letter are not significantly different at $p \leq 0.05$. T0: Control; PO: Palm oil; MO: *Mbuja*; oil; CO: Corn oil. N= 6 rats per group.

Body weight and body weight gain are evaluated to witness acceptability of food submitted for each regimen. The body weight increased in each group after 50 days. Compared to control group (T0) (12.84±1.16%), and CO (22±3%) the body weight gain on MO (8.41±2.21%) has shown less value, while in PO (16.34±1.54%) the increase is very important. This increase of body weight observed can be a witness of acceptability and tolerance to the regime submitted.

From these investigations, it appears that the different diets applied did not lead to any variation ($p<0.05\%$) in the viscerosomatic index of the kidney and heart compared to the control (T0). This can be explained by the fact that at 7 months of age, the rats have completed their growth hence the stability in the VSI ratio. The different diets applied lead to an increase in the hepatosomatic index (HSI). This is due to liver hypertrophy as a result of increased biochemical activities to regulate blood lipid flow [18]. Duncan's test indicated no significant difference ($p<0.05$) in hepatosomatic index (HSI) values of rats in the MO (4.06±0.31%), PO (3.75±0.27%) and T0 (3.72±0.6%) groups. MO and CO (4.44±0.54%) have higher values than PO. This high value can be explained by the high level of polyunsaturated fatty acids and crude phenolic compounds which can act as antioxidant and neutralize toxins. Toxins will be then destroy by liver and expulse by kidneys. That is why HSI and VSI increase compared to T0. The HSI value obtained with PO and T0 group are close to that found by Kritchesky *et al*. [19] (3.08±0.75%) on two-month-old Sprague Dawley rats fed with 10% palm oil in the diet for 3 weeks. Significant ($p<0.05$) and negative correlations are noted between body gain and VSI of Right kidney ($R= -0.85$) and Left kidney ($R= -0.70$). This means that the increase of body mass doesn't lead the increase of kidney mass. In the other side, significant ($p<0.05$) and positive correlation is observed between HSI and VSI of heart ($R= 0.88$) and left kidney ($R= 0.74$). These correlations could be explained by the fact that intense activity of liver is correlated heart and left kidney activity. The consumption of *mbuja* oil did not result in a significant increase of heart, liver and kidneys mass of rats compared to T0. All this suggests a non-toxic effect of this oil and good organ function.

3.2. Evaluation of Renal Dysfunction Factors

The determination of renal dysfunction factors is carried out in order to assess the effects of *mbuja* oil consumption on the kidneys. Creatinine is a biochemical parameter considered to be the most effective endogenous marker in the diagnosis and treatment of renal pathologies [20]. Renal dysfunction in rats tested for 50 days was assessed by measuring serum creatinine and protein levels.

3.2.1. Serum Creatinine Level

The determination of creatinine in the serum of rats is carried out in order to assess whether if consumption of *mbuja* oil causes damage to renal function. The increase in creatinine concentration is associated with the risk of renal failure [21]. Figure 1 shows the serum creatinine levels of rats in the different experimental groups.

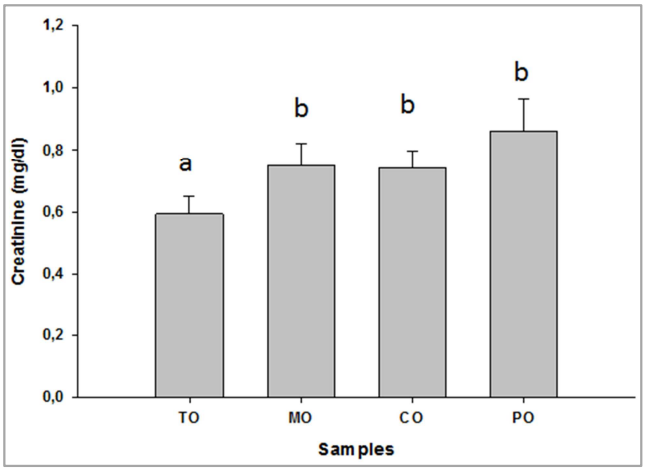

Figure 1. *Serum creatinine levels.*

Histograms with the same superscript letters are not significantly different ($p<0.05$).
T0: control group; MO: *mbuja* oil group; CO: maize oil group; PO: palm oil group. N= 6 rats per group.

The figure 1 shows that the serum creatinine level of rats increase according to the regimen. From 0.59±0.06 mg/dl in T0, this value increases by 27% (MO); 25% (CO); 46% (PO) after 50 days of feeding with proposed diets. These values are 0.75±0.07 mg/dl; 0.74±0.06 mg/dl and 0.86±0.11 mg/dl respectively for MO, CO and PO diets. Creatinine value found with MO is not far from the value (0.80±0.12 mg/l) obtained by Doumta and Tchiegang [22] by feeding rats with *daddawa* oil. The serum creatinine level of rats fed with MO, CO and PO diets were not significantly different ($p<0.05$). The slight differences in concentrations observed may be due to the fact that biochemical reaction change according to the metabolism. Research of correlation shows positive and significant ($p<0.05$) correlation ($R= 0.88$) between creatinine concentrations and heart's VSI. This confirms existence of correlation between heart and creatinine level. *Mbuja* oil did not affect the serum creatinine concentration of rats.

3.2.2. Serum Protein Levels

The purpose of the protein assay is to assess the influence of fat consumption on protein metabolism. Figure 2 shows the protein levels of each group of rats after 50 days of feeding.

Regardless of the diet, the protein concentration varied constantly compared to the control except in the case of CO (50.6±1.51 g/l). There is significant difference ($p <0.05$) between the serum protein concentrations of the T0 (49.00±2.52 g/l) control group and those from MO (52.33±1.53 g/l) and PO (56.33±3.60 g/l) groups. These results are not far from those found by Doumta and Tchiegang [22] on *daddawa* oil (51.20±1.82 g/l), but lower than those observed by Moundipa *et al.,* [23] (127.87±3.36 g/l) and Tchankou Leudeu [24] (65.50±5.85 g/l) in rats fed with palm oil diet. Research of correlation between serum protein and creatinine levels shows that there is significant and positive correlation ($R = 0.68$; $p < 0.05$) between these two biochemical parameters. The serum protein level is therefore related to the blood creatinine level and expresses the activity of the kidneys to evacuate urea from protein metabolism. The consumption of *mbuja* and maize oils has no effect on serum protein.

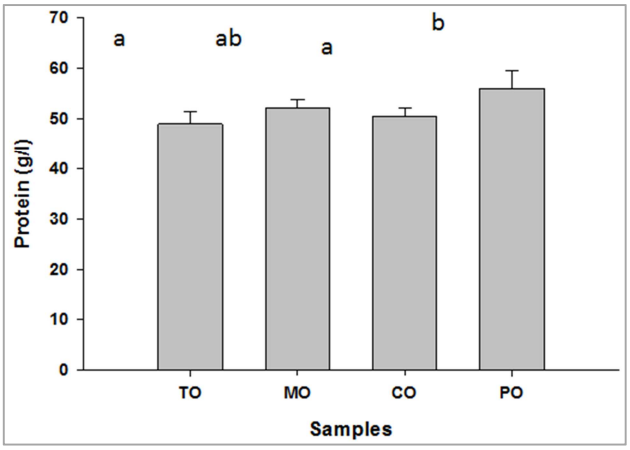

Figure 2. *Serum protein levels.*

Histograms with the same superscript letters are not significantly different ($p<0.05$).
T0: control group; MO: *mbuja* oil group; CO: maize oil group; PO: palm oil group. N= 6 rats per group.

3.3. Evaluation of Liver Dysfunction Factors

The determination of liver dysfunction factors is carried out in order to assess the effects of *mbuja* oil consumption on the liver. Liver dysfunction in rats tested for 50 days was assessed by measuring ALT, AST and bilirubin levels.

3.3.1. Serum Alanine Aminotransferase (ALAT) and Aspartate Aminotransferase (ASAT) Levels

Figure 3 shows the serum ALAT and ASAT concentration of different groups of rats tested.

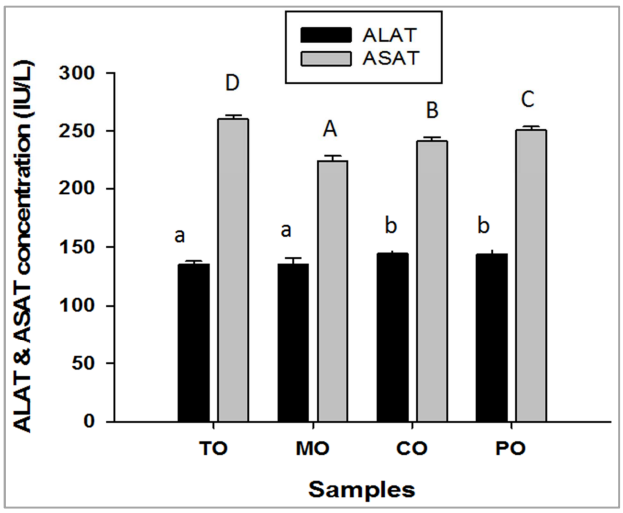

Figure 3. *Serum ALAT and ASAT levels.*

Histograms with the same superscript and same character of letters are not significantly different ($p<0.05$).
T0: control group; MO: *mbuja* oil group; CO: corn oil group; PO: palm oil group. N= 6 rats per group.

The serum alanine aminotransferase (ALAT) level is measured in order to show the state of liver function. In general, an elevated serum ALAT level is mainly considered as an indicator of parenchymal liver disease. ALAT is the best indicator for diagnosing hepatobiliary disease than aspartate aminotransferase [25]. It is a normal enzyme of the liver and cardiac cells. It is found in the blood when the liver and heart are damaged.

According to Duncan's test, there is no significant difference ($p<0.05$) between the serum ALAT values of CO (144.67±2.85 IU/l); PO (144.00±2.29 IU/l). In the same way MO (134.66±2.68 IU/l) and T0 (135.33±2.52 IU/l) are so close. However, there is a significant decrease ($p<0.05$%) in MO value compared to T0. Similar results were found by Doumta and Tchiegang [22] on *daddawa* oil with lower ALAT value (112.40±8.93 IU/l). Decrease in ALAT value observed with MO would reflect a good state of liver and heart function. MO oil would contribute to the good functioning of the liver and heart compared to CO and PO.

The determination of ASAT is useful in the diagnosis and monitoring of hepatobiliary diseases, myocardial infarction and skeletal muscle damage [25]. This enzyme is present in the blood when the liver and heart are damaged. Figure 3 compares the serum ASAT levels of the different groups of oils tested. Results in figure 3 show that the serum ASAT level remains almost constant during the experiment. At the beginning of the experiment the level (T0) is 260.20±3.6 IU/l. A mean level of 243 IU/l was obtained after 50 days of feeding with different diets. Significant difference ($p<0.05$) is observed between ASAT values of MO (224.0±4.58 IU/l), CO (241.33±3.21 IU/l), PO (250.67±3.05 IU/l) and T0 for the proposed oil. These values are not far from those found by Doumta and Tchiegang [22] on *daddawa* (248 IU/l) and corn oil (240 IU/l). However, there was a decrease in MO, CO and PO compared to T0. This decrease would reflect a good functioning of the liver, heart and kidney. For this purpose, negative and significant ($p<0.05$) correlation is observed between VSI of kidney (left R= -0.95 and right R= -0.86) and ASAT concentration. In contrary, positive and significant ($p<0.05$) correlation (R= 0.74) is noted between body mass gain and ASAT concentration. These correlation observed can be explained by the fact that skeletal muscle damage produced by physical stress during the experimental period, are eliminated by kidney activities. That is why correlation is observed with kidneys and body mass gain. Elimination of damaged cells will help to protect liver and heart against intoxication. Thus, consumption of *mbuja* oil may help to protect liver and heart cells more than CO and PO.

3.3.2. Importance of ASAT/ALAT Ratios

The increase in the level of ASAT in relation to ALAT (Ritis quotient: ASAT/ALAT) is useful in detecting liver damage. A quotient less than 1 indicates a benign liver injury and is found in diseases of an inflammatory nature. A quotient greater than 1 indicates severe liver disease often accompanied by necrosis [22]. Figure 4 compares the ASAT/ALAT ratios of different diets.

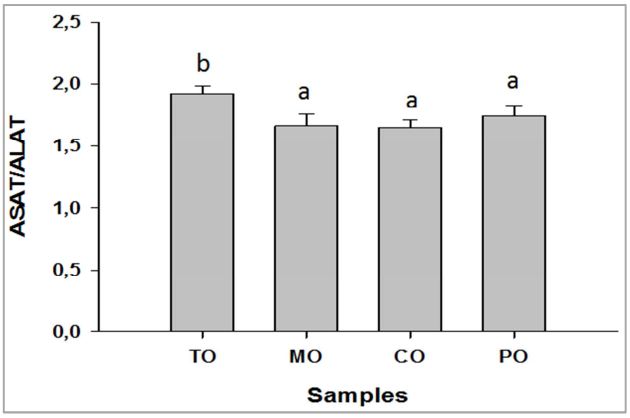

Figure 4. *ASAT/ALAT ratios according to diet.*

Histograms with the same superscript letters are not significantly different ($p<0.05$).
T0: control group; MO: *mbuja* oil group; CO: corn oil group; PO: palm oil group. N= 6 rats per group.

In this study, the ASAT/ALAT ratio are 1.65; 1.67; 1.74 and 1.92 for the MO, CO, PO and T0 respectively. From these ratios, it can be seen that the rats in MO group were more likely to show good state liver and heart. Analysis of ALAT levels revealed that there is no damage to liver function (Figure 3). The cause of the decrease of ASAT/ALAT ratio would therefore justify the quality of liver and heart comparing to other groups [26].

3.3.3. Serum Bilirubin Levels

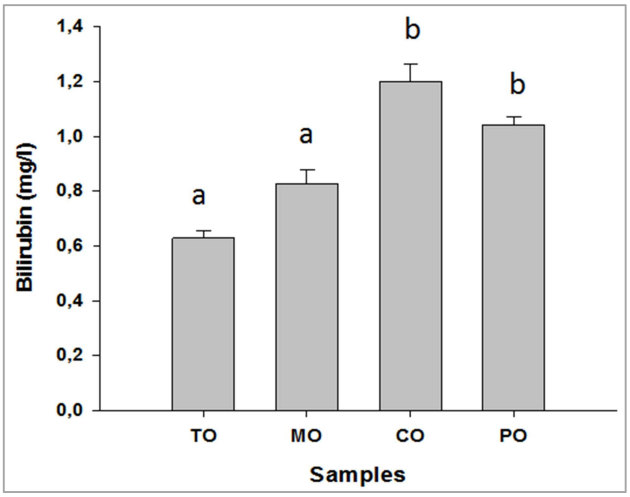

Figure 5. *Serum bilirubin level.*

Histograms with the same superscript letters are not significantly different ($p<0.05$).
T0: control group; MO: *mbuja* oil group; CO: corn oil group; PO: palm oil group. N= 6 rats per group.

Figure 5 shows the serum bilirubin concentrations of rats for different experimental groups. Bilirubin is a bile pigment originating from the physiological destruction of aged red blood cells (haemoglobin is degraded by hepatocytes) or

haematopoiesis (excessive synthesis of haemoglobin or premature destruction of young red blood cells) or from the turnover of non-haematological metallo-porphyrins (cytochromes, catalases) [27].

The serum bilirubin level is measured in order to highlight the state of proper functioning of the liver. Its serum concentration increases during hepatitis.

The bilirubin concentrations is ranged from 0.63±0.02 mg/l in the T0 group to 1.23±0.06 mg/l in the CO group. No significant difference ($p < 0.05$) is observed between bilirubin concentrations of the MO (0.82±0.05 mg/l) and T0 (0.63±0.02 mg/l). Consumption of *mbuja* oil could therefor ensure, on physiological level, a reduction of red blood cell ageing, normal haemoglobin synthesis, protection of juvenile red blood cells or increase the efficiency of non-haematological metallo-porphyrins (cytochromes, catalases) resulting in the reduction of bilirubin production [27]. The low bilirubin concentration obtained with MO diet is evidence of good liver cell function and confirms the results of the good liver condition noted in ALAT and ASAT assay. This is reason why significant correlations are observed between HSI and bilirubin (R= 0.65), but also between ALAT and Bilirubin (R= 0.77). This justifies the assertion that MO is used in traditional medicine for its hepatoprotective effects.

4. Conclusion

The results of this study revealed no toxicity after feeding rat with *mbuja* oil during 50 days. The determination of organs dysfunction factors showed good function of kidney, heart and liver. Consumption of *mbuja* oil protects and improves kidneys, liver, heart function. This study confirms the hepatoprotective activity *of mbuja* and justify its use in traditional medicine in treatment of inflammatory cardiovascular and chronic hepatic diseases. Further studies are needed to assess and identify molecules which are responsible for these activities.

Abbreviations

T0: Control;
PO: Palm oil;
MO: Mbuja oil;
CO: Corn oil;
ALAT: Alanine aminotransferase;
ASAT: Aspartate aminotransferase;
HSI: Hepatosomatic index;
VSI: Viscerosomatic index.

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