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Effects of Aqueous Extract of *Cola anomala* Nuts on Hyperglycemia and Associated Complications.

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ABSTRACT

Hyperglycemia and its complications represent a major public health problem, and are associated with a high mortality rate. The objective of this study was to evaluate the effect of aqueous extract of *Cola anomala* nuts on hyperglycemia and some associated complications. The effect of the extract on postprandial hyperglycemia was evaluated in normoglycemic rats using the oral glucose tolerance test. Subsequently, the effect of the extract on fasting blood glucose was evaluated in an animal model of hyperglycemia induced by streptozotocin. The extract at both doses (200 and 400 mg/kg.bw (mg/kg. bodyweight)) caused a significant reduction (P= 0.003) of postprandial hyperglycemia with percentages of variations of -4.5% and -14.4% against that of glibenclamide (-30.6%). The extract significantly lowered fasting blood glucose with variations of -68.09% and -65.98% respectively compared to Glibenclamide (-64.7%). There was an increase in superoxide dismutases and catalase activities at the renal level and a decrease in plasma and hepatic malondealdehyde and nitric oxide levels with the extract at different doses. The extract at the dose of 400 mg/kg.bw showed an improvement of the globular filtration as plasma creatinine concentrations were found to be decreased, as well as a hepatoprotective effect through the significant reduction of plasma aspartate amino transferase (AST) and alanine amino transferase (ALT) activities. In conclusion, the aqueous extract of *Cola anomala* nuts has a beneficial effect on the control of hyperglycemia and in the prevention of oxidative stress which is strongly involved in the development of microvascular complications of diabetes.

Keywords: *Cola anomala*, hyperglycemia, microvascular complications.

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INTRODUCTION

Diabetes is a metabolic condition characterized by chronic fasting hyperglycemia disrupting carbohydrate, and consequently lipid and protein metabolisms[1]. This condition is due to a failure of insulin secretion, action or both associated abnormalities, resulting in specific complications affecting vital organs and tissues[1]. Diabetes is experiencing a very significant expansion with 463 million diabetic people in the world and a prediction of 700 million by 2045 if no action is taken[2]. Cameroon is no exception with 615,300 diabetics in 2020[3].

Hyperglycemia whether postprandial or fasting is the major characteristic of diabetes and is strongly implicated in the development of associated complications. Indeed, high consumption of high energy foods leads to postprandial state, which requires mobilization of the regulatory system to prevent the occurrence of postprandial hyperglycemia[4]. Postprandial hyperglycemia results from the persistent and prolonged increase in blood glucose and it depends on the digestibility of carbohydrates as well as the regulatory capacity of pancreatic cells[5]. In the diabetic patient, there is an overproduction of reactive oxygen species (ROS) on the one hand and a decrease in antioxidants on the other hand, this imbalance generates a state of oxidative stress which is at the origin of micro and macro-angiopathic complications of diabetes[6]. Abnormally high concentrations of circulating glucose can induce oxidative stress through various mechanisms. In fact, chronic fasting hyperglycemia can lead to over activation of the electron transporter chain in the mitochondria, resulting in an overproduction of superoxide anions. The latter not only increases the production of ROS but also reduces the antioxidant potential. However, impairment of the mitochondrial respiratory chain is the primary cause of increased oxidative stress and can also cause damage to organs such as the liver, kidneys and heart. Management strategies for diabetes and its complications are mostly oriented towards lifestyle changes by adopting a balanced diet[7]. In addition, regular exercise significantly improves insulin-mediated glucose transport to the muscles[8]. However, when these lifestyle changes are not sufficient for the intervention of this disease, pharmacological management through oral antidiabetic drugs (OADs) and insulin therapy is used[9].

The excessive cost of these anti-diabetic agents and the lack of medical infrastructure in Africa lead people to turn to traditional medicine. Thus, several medicinal plants have shown their beneficial effects in the management of diabetes either by their capacity to reduce hyperglycemia or by the reduction of oxidative stress which is strongly involved in the development of its complications. Recent studies on *Cola anomala*, a plant of the Cameroon pharmacopoeia revealed that the aqueous extract of its nuts has a high content of alkaloids and phenolics

especially flavonoids and also has good antioxidant potential *in vitro*[10,11]. However, its anti-diabetic activity remains unknown. The objective of this study was to evaluate the effect of the aqueous extract of *Cola anomala* nuts on hyperglycemia and associated complications.

MATERIALS AND METHODS

Collection, identification and preparation of plant material

The plant material used was *Cola anomala* nuts harvested in *Bandjoun* (West Region, Cameroon). They were identified at the National Herbarium of Cameroon in comparison with the sample of *Cola anomala K. Schum* collected by B.A. *Nkongmeneck* N° 113 of the specimen of the herbarium collection N°48706/HNC (YA). Once at the Laboratory of Nutrition and Nutritional Biochemistry (LNNB), they were cleaned, shade-dried to a constant weight, then ground in a blender, weighed and an aqueous extraction was performed.

Preparation of the aqueous extract of *Cola anomala* nuts (EACA).

Powdered *C. anomala* nuts were macerated in distilled water (ratio 1:6 w/v) for 24h after which the mixture was filtered using number 3 whatman paper. The filtrate obtained was placed in an oven to evaporate the solvent at 40°C for 72h. The obtained extract was stored at room temperature for further use.

Design of experimental study

Experimental animals were provided by the animal house of the LNNB of the Department of Biochemistry, Faculty of Science, University of Yaounde I. None of the animals had been subjected to previous experiments and had showed no signs of abnormalities. The animals were placed in standard cages under recommended conditions. They were subjected to a 12-hour day-night cycle with good ventilation and natural light. They had access *ad libitum* to running tap water and a standard diet. The experimental protocol and the care of the laboratory animals were carried out in accordance with the standard ethical guidelines of the Joint Institutional Review Board for Animal and Human Bioethics, CRFD-SVSE of the University of Yaounde I.

Evaluation of the effect of the extract on postprandial hyperglycemia

The effect of the extract on postprandial hyperglycemia was evaluated through the glucose tolerance test (OGTT) according to the method of Oguanobi *et al.*[12] with slight modification. The rats were fasted for 12 hours and were randomly divided as follows:

A Negative Control (NC) group: receiving water.

A Positive Control (PC) group: receiving glucose solution (2 g/kg.bw).

A Test group 1 (EACA200): receiving glucose solution (2 g/kg.bw) + aqueous extract *Cola anomala* (200 mg/kg.bw).

A Test group 2 (EACA400): receiving glucose solution (2 g/kg.bw) + aqueous extract *Cola anomala (*400 mg/kg.bw).

A reference group (REF): receiving glucose solution (2 g/kg.bw) + Glibenclamide (dose of 5 mg/kg.bw)

The extracts, water and Glibenclamide were administered by gavage through esophageal tube immediately after taking fasting blood glucose. Thirty minutes later, a glucose solution (2 g/kg.bw) was administered (by gavage) in all groups except the NC group. Blood glucose levels were assessed using a glucometer and *One Touch* GOD-POD strips using whole blood collected from the tail vein of rats at 30, 60, 90, 120, 150, and 180 minutes after glucose administration. The results were expressed as glucose variations according to the formula:

Blood glucose variation $= \frac{Glycemia\ (Tx) - Glycemia\ (T0)}{Glycemia\ (T0)}$; T0: Blood glucose at time t= 0, Tx: Blood glucose after every 30 min.

Evaluation of the effect of the extract on induced hyperglycemia and some associated complications

Experimental protocol

Hyperglycemia was induced in rats following the protocol of Al-Shamaony *et al.*[13] with modifications by intraperitoneal injection of streptozotocin (STZ) at a dose of 45 mg/kg.bw (dissolved in 100 mM citrate buffer, pH 4.5 150 mM NaCl). One hour after STZ injection, the animals were given glucose water (20%), to avoid hypoglycemic shock following STZ administration. After 24 hours, the blood glucose levels of the rats were taken and the animals with blood glucose levels greater than or equal to 200 mg/dL were diagnosed

as hyperglycemic and allocated for further experimentation as follows:

Group I (NC): normoglycemic rats receiving water by gavage.

Group II (PC): untreated hyperglycemic rats receiving water by gavage.

Group III (EACA 200): hyperglycemic rats receiving *Cola anomala* aqueous extract by gavage at 200 mg/kg.bw.

Group IV (EACA 400): hyperglycemic rats receiving *Cola anomala* aqueous extract by gavage at a dose of 400 mg/kg.bw.

Group V (Reference) hyperglycemic rats receiving glibenclamide by gavage at a dose of 5 mg/kg.bw.

The number of rats in each group was equal to 5. The maximum volume of administration was set at 5ml. All solutions (water/extract/glibenclamide) were administered daily. During the

experiment, blood glucose levels were taken for each group at time intervals of two weeks for a 28 days experimentation period. Percentage changes were calculated according to the formula:

Variation of blood glucose levels (%)= $\frac{Glycemia\ (GX) - Glycemia\ (G0)}{Glycemia\ (G0)} x\ 100$; G0: Blood glucose at day = 0, Gx : Blood glucose at a given day.

I.5.2. Sacrifice of animals and preparation of biological samples.

At the end of the experiment, the animals were fasted for 12 hours and sacrificed. Blood was collected into EDTA tubes and centrifuged for the preparation of plasma and hemolysate. Homogenates of 10% of the organs were prepared in NaCl solution (0.9%). All prepared biological samples were stored at -20°C.

Evaluation of stress markers.

The effects of the extract on markers of oxidative stress were evaluated by determining the activity of antioxidant enzymes (catalase, SOD) and through the determination of prooxidant levels of malondyaldehyde (MDA) and nitric oxide (NO).

SOD activity was determined in hemolysate of red blood cells, liver and kidney according to the method of Misra and Fridovish[14].

Catalase activity was determined in hemolysate, liver and kidney according to the method of Sinha[15]. For this activity, protein concentrations were also determined according to the method described by Gornall *et al.*[16].

Lipid peroxidation was assessed in plasma, liver and kidney by the determination of Malondyaldehyde (MDA) according to the method of Yagi[17].

Determination of nitric oxide radical in plasma, liver, kidney was done according to the method of Manish *et al.*[18]

Evaluation of markers of liver and kidney toxicity.

The activity of plasma transaminases (Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) was determined according to the method described by Reitman and Frankel[19].

Plasma creatinine levels were determined according to the method of Bartels *et al.*[20].

Statistical Analysis

Results were expressed as mean ± standard deviation. The change in blood glucose levels over time (minutes) was plotted as a percentage. *SPSS (Statistical Package for Social Science)* version 20.0 for *Windows* was used for statistical analysis of the results. One factor *Analysis Of Variance* (ANOVA) followed by LSD (*Least Significant Difference)* were used to compare the means of different groups. *Microsoft Excel* software was used to process the data and plot the graphs. The results were significant for $P \leq 0.05$.

RESULTS

Effects of aqueous extract of *Cola anomala* nuts on hyperglycemia induced by a high dose of glucose orally.

The effects of the extract on postprandial hyperglycemia were expressed as percentage changes in blood glucose levels and mean blood glucose levels (Table 1). Thirty minutes after glucose administration, there was an increase ($P<0.05$) in blood glucose levels in all groups with a mean blood glucose level of 150 mg/dL compared to the NC group (group that received only distilled water). The extract at the dose of 200 mg/kg.bw acted essentially by significantly limiting the glycemic peak obtained after 30 min compared to the positive control while the extract at the 400 mg/kg.bw lowered progressively and significantly the blood glucose with percentages of variations of 79.3%; 7%; -8.8%, -9.6%, and -14.4% at time (min) T30, T90, T120, T150 and T180 respectively compared to the PC group. Only at T60 was the REF group (glibenclamide treated group) found to be significantly more effective than the extract at 400 mg/kg.bw. At 150 min, no significant difference was noted between the 200mg dose and the reference group although the reference group was found to be significantly more effective than the extract at 200 mg/kg.bw, throughout the experiment

Table 1: Change in blood glucose levels after administration of glucose, aqueous extract of C. *anomala* nuts and glibenclamide to normal rats.

Groups		Time (minutes)						
		0	**30**	**60**	**90**	**120**	**150**	**180**
NC	Blood glucose (mg/dl)	75,2±2,0	76,7±3,9	66,7±4,0	59,5±4,0	57,7±5,5	54,7±2,5	50,2±0,7
	Change (%)	(0)	(2,4)[a]	(10,9)[a]	(14,4)[a]	(23,0)[a]	(27.2)[ac]	(23,6)[a]
PC	Blood glucose (mg/dl)	67±3,2	146,7±3,2	116,7±3,8	85,2±3,2	76,2±5,2	74±9,6	71±3,5
	Change (%)	(0)	(119,)[b]	(75,1)[b]	(19,7)[b]	(14,29)[b]	(10,4)[b]	(5,4)[b]
AECa200	Blood glucose (mg/dl)	75±4,3	127,2±5,6	104,2±5,4	90,5±4,3	87,2±12,2	78,4±13,5	78,7±1,0
	Change (%)	(0)	(71,4)[c]	(40,8)[b]	(16,3)[b]	(18,1)[b]	(4,7)[b]	(4,5)[b]
AECa400	Blood glucose (mg/dl)	74,2±6,0	133±6.4	105,7±6,6	80±6,0	67,5±4,3	66,7±8,7	58,4±4,0
	Change (%)	(0)	(79.3)[cd]	(42,8)[b]	(7,0)[a]	(-8,8)[a]	(-9,6)[a]	(-14,4)[a]
REF	Blood glucose (mg/dl)	75,2±3,3	195,3±9,2	67,7±9,8	62±7,3	55,7±7,8	46,2±16,1	43,2±12,0
	Change (%)	(0)	(26,5)[d]	(-6,6)[a]	(-11,9)[a]	(-24,6)[a]	(-37,3)[c]	(-30,6)[a]

Values are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; EACA200: hyperglycemic rats treated with 200 mg/kg.bw of C. anomala; EACA400: hyperglycemic rats treated with 400 mg/kg.bw of C. anomala; REF: Hyperglycemic rats treated with 5 mg/kg.bw of glibenclamide. The values assigned with different letters (a, b, c, d) are significantly different (P<0.05)

Effect of the extract on hyperglycemia in rats after treatment with streptozotocin (STZ).

The effects of the extract on fasting hyperglycemia were expressed as mean blood glucose levels and percentage changes (Table 2). One day after STZ administration to the rats, a significant increase in blood glucose was observed with a maximum of 400 mg/dL in treated rats compared to non-treated rats (NC group). The 400 mg/kg.bw extract significantly lowered blood glucose levels in the first two weeks with a percentage change of -70.6%, and in the fourth week by -65% compared to the PC group. There was no significant difference between the extract at 200 mg/kg.bw and the reference, while at 14 days, the extract at 400 mg/kg.bw was already more effective than the reference.

Table 2: Variations in fasting blood glucose after STZ administration in rats.

		Days		
Groups		**1**	**14**	**28**
NC	Blood glucose (mg/dL)	101,8±3,2	97,2±2,5	91±1,1
	Variation (%)	(0)	(-2,7%)[a]	(-29,1%)[a]
PC	Blood glucose (mg/dL)	299,0±5,5	183,1±4,4	143,5±6,1
	Variation (%)	(0)	(-39,6%)[b]	(-51,2%)[b]
EACA200	Blood glucose (mg/dL)	319,2±9,2	177±7,3	86,8±5,1
	Variation (%)	(0)	(-56.3%)[bc]	(-68 ,0%)[c]
EACA400	Blood glucose (mg/dL)	304,7±2,4	97,7±3,5	109,7±6,6
	Variation (%)	(0)	(-70,6%)[c]	(-65,9%)[c]
REF	Blood glucose (mg/dL)	374±1,16	213,4±6,84	117,1±6,37
	Variation (%)	(0)	(-40,6%)[b]	(-64,7%)[c]

Values are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; EACA200: hyperglycemic rats treated with 200 mg/kg.bw of C. anomala; EACA400: hyperglycemic rats treated with 400 mg/kg.bw of C. anomala; REF: Hyperglycemic rats treated with 5 mg/kg.bw of glibenclamide. The values assigned with different letters (a, b, c, d) are significantly different (P<0.05).

Effect of the extract on some markers of oxidative stress.

Figure 1 shows the effect of EACA on superoxide dismutase (SOD) activity. No significant difference was noted in erythrocytes between the different groups. However, SOD activity was significantly lower in the liver and kidney of rats in the PC group compared to the NC group. Compared to PC, the extract at both doses (200 and 400 mg /kg.bw respectively) showed significantly higher SOD activities in kidney and liver. Except in kidney where the extract at 200 mg/kg.bw showed significantly higher activity, no significant difference was noted between the extracts and the reference.

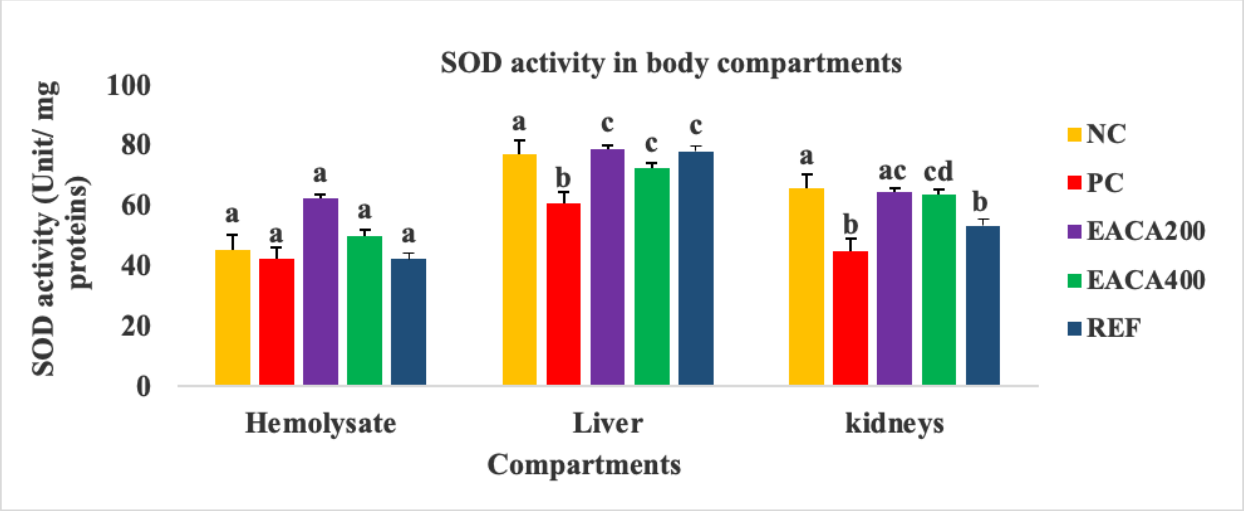

Figure 1: Influence of aqueous extract of *Cola anomala* nuts on SOD activity.

Values are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; EACA200: hyperglycemic rats treated with 200 mg/kg.bw of C. anomala; EACA400: hyperglycemic rats treated with 400 mg/kg.bw of C. anomala; REF: Hyperglycemic rats treated with 5 mg/kg.bw of glibenclamide. The values assigned with different letters (a, b, c, d) are significantly different (P<0.05).

The effect of the extract on catalase activity is shown in Figure 2. These results showed a significant decrease in catalase activity in erythrocytes, liver and kidney in the PC group compared to NC. The extract at both doses showed significantly higher renal catalase activities than the reference, while no significant difference was noted in hemolysate and liver.

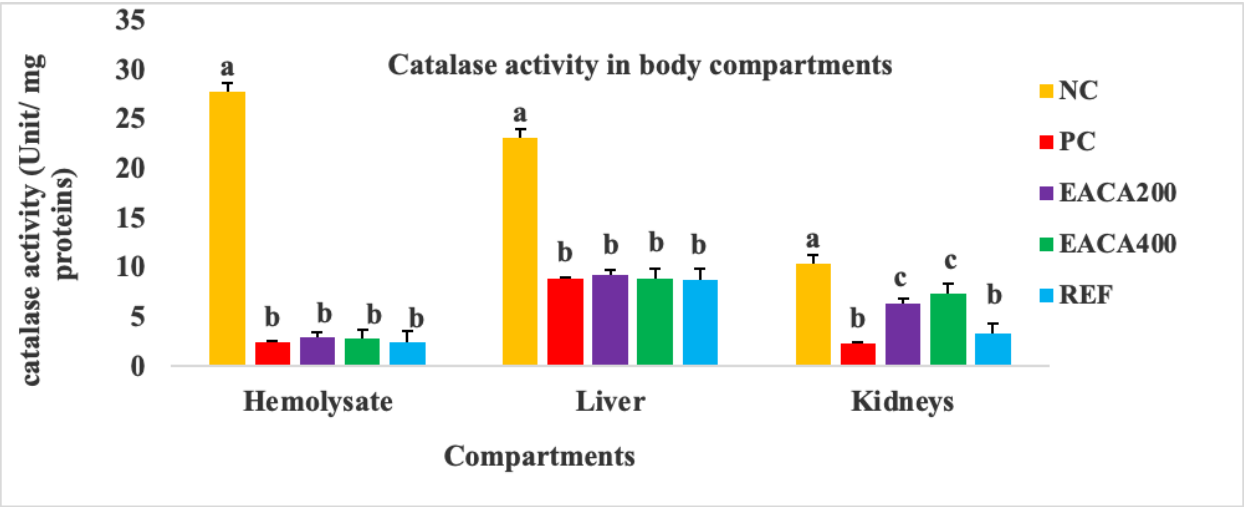

Figure 2: Influence of aqueous extract of *Cola anomala* nuts on catalase activity.

Values are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; EACA200: hyperglycemic rats treated with 200 mg/kg.bw of C. anomala; EACA400: hyperglycemic rats treated with 400 mg/kg.bw of C. anomala; REF: Hyperglycemic rats treated

with 5 mg/kg.bw of glibenclamide. The values assigned with different letters (a, b, c, d) are significantly different (P<0.05).

Figure 3 shows the effect of the extract on MDA levels in some compartments. There was a significant elevation in plasma hepatic and renal MDA levels in the PC group compared to the NC group. Compared to PC, at 200 mg/kg.bw, the extract led to significantly lower levels of MDA in plasma, liver and kidney. However, there was no significant difference between the extract at 400 mg/kg.bw and PC in plasma and kidney. nor was there a difference between the 400 mg/kg.bw extract and the reference in the liver, All the same, at 200 mg/kg.bw, MDA levels were significantly lower than the reference in the liver.

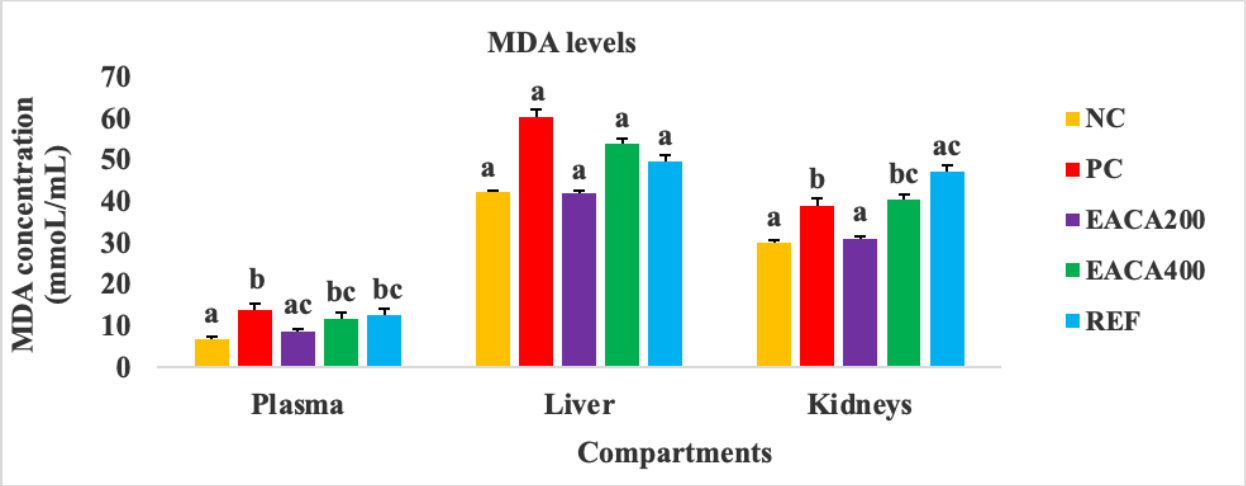

Figure 3: Effect of aqueous extract of *Cola anomala* on plasma, liver and kidney MDA levels.

Values are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; EACA200: hyperglycemic rats treated with 200 mg/kg.bw of C. anomala; EACA400: hyperglycemic rats treated with 400 mg/kg.bw of C. anomala; REF: Hyperglycemic rats treated with 5 mg/kg.bw of glibenclamide. The values assigned with different letters (a, b, c, d) are significantly different (P<0.05).

The results of the level of NO in body compartments are represented in Figure 4. No significant difference was observed between the different groups in the liver and kidney. There was a significant (P<0.05) increase in NO levels in the liver of the PC group compared to the NC group. However, *Cola anomala* aqueous extract at both doses significantly (P=0.003) decreased the NO level in the liver compared to the PC group. Levels were nonetheless lower in rats treated with the reference drug compared to those treated with the extracts.

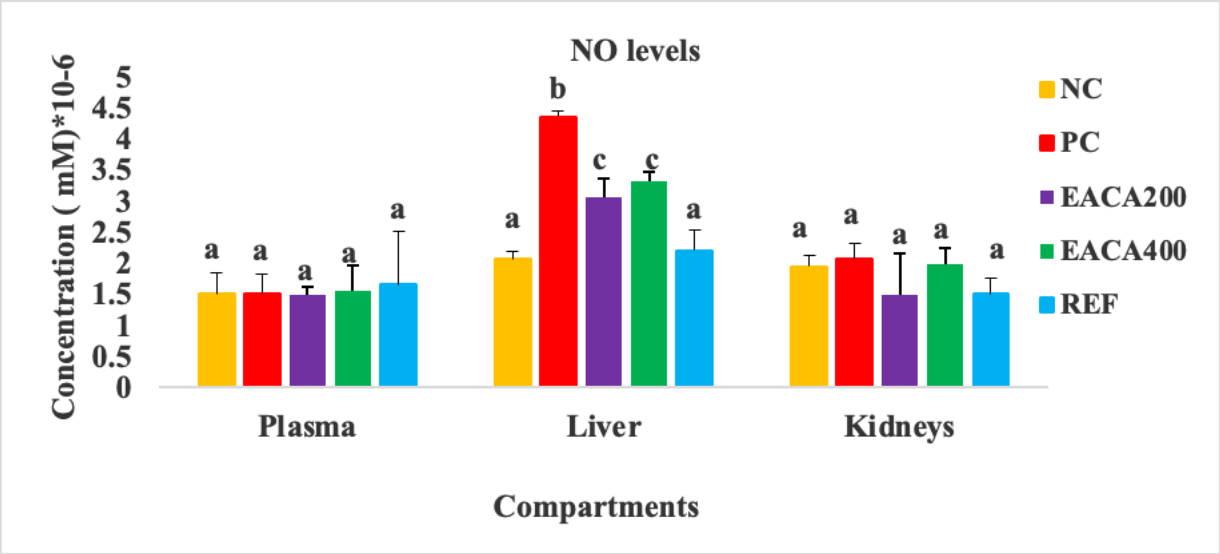

Figure 4: **Effect of aqueous extract of *Cola anomala* on plasma, hepatic and renal nitric oxide (NO) production.**

Values are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; EACA200: hyperglycemic rats treated with 200 mg/kg.bw of C. anomala; EACA400: hyperglycemic rats treated with 400 mg/kg.bw of C. anomala; REF: Hyperglycemic rats treated with 5 mg/kg.bw of glibenclamide. The values assigned with different letters (a, b, c, d) are significantly different (P<0.05).

The results in Figure 5 represent the activity of transaminases in the plasma of experimental rats. Regarding ALT activity, no significant difference was noted between the NC, PC and extract groups; however, the activity was significantly higher in the reference group compared to all the other groups. There was a significant elevation of AST activity in the PC group compared to the NC group. On the other hand, the aqueous extract of *Cola anomala* nuts at the dose of 400 mg/kg.bw led to a significant decrease of (43.72 unit/L) in its activity compared to that of PC group (50.22 unit/L). The extract at 400 mg/kg.bw significantly reduced the activity of AST compared to the reference. No significant difference is observed with the transaminase ratio.

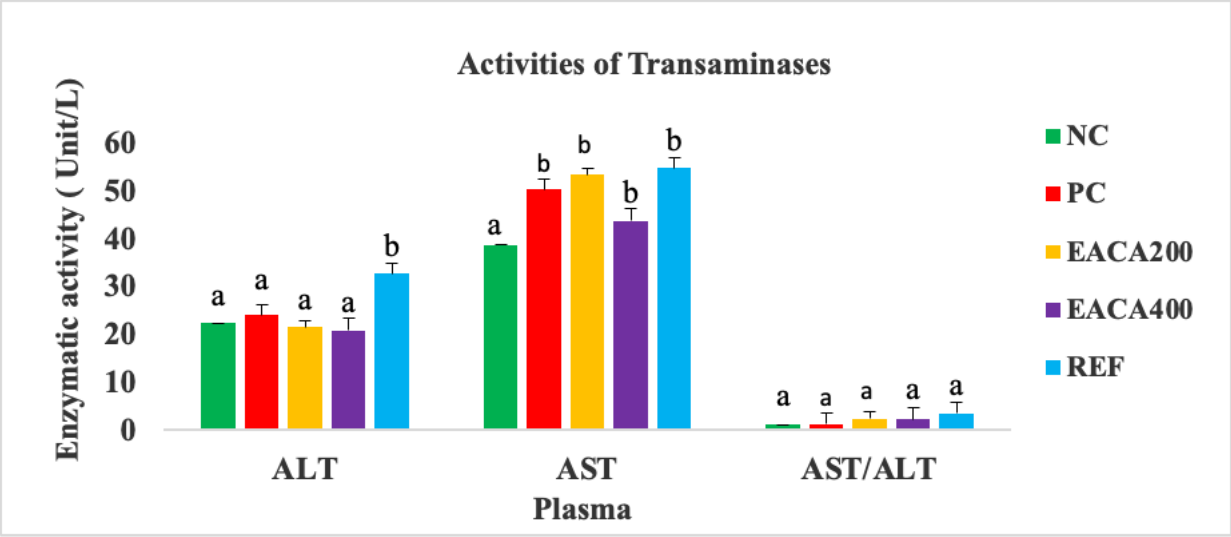

Figure 5: Effect of aqueous extract of *Cola anomala* nuts on markers of liver function.

Values are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; EACA200: hyperglycemic rats treated with 200 mg/kg.bw of C. anomala; EACA400: hyperglycemic rats treated with 400 mg/kg.bw of C. anomala; REF: Hyperglycemic rats treated with 5 mg/kg.bw of glibenclamide. The values assigned with different letters (a, b, c, d) are significantly different (P<0.05).

Figure 6 shows the influence of extract on plasma creatinine concentration. Compared to the NC group, there was a significant increase in creatinine concentration in the PC group. The extract at the dose of 400 mg /kg.bw significantly decreased the creatinine concentration (1.79 mg/dL) compared to the PC group (2.67 mg/dL). The reference group resulted in a decrease in creatinine compared to the extract at the dose of 200 mg/kg. bw ($p<0.05$).

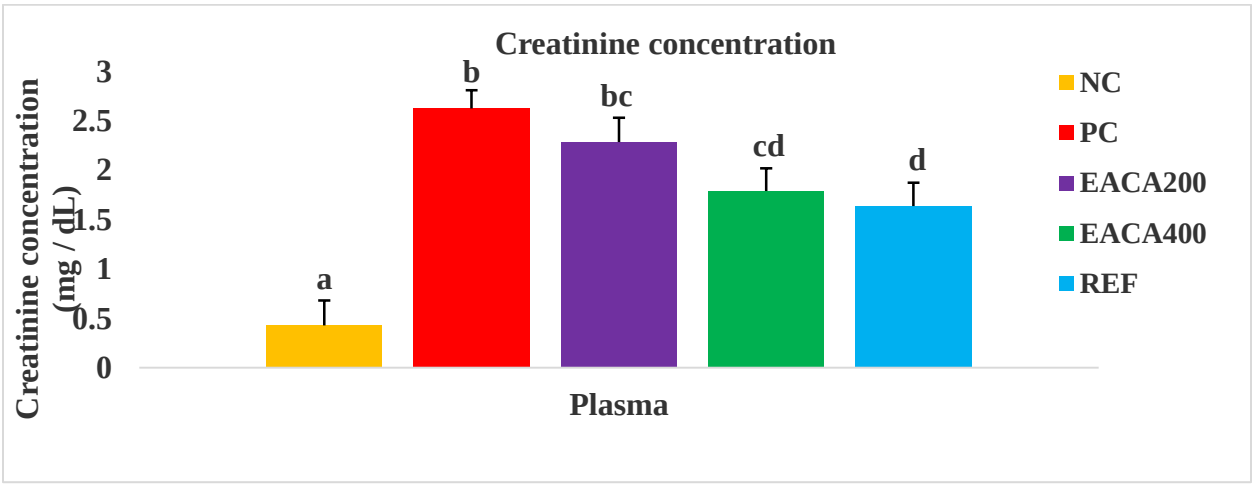

Figure 6: Effect of aqueous extract of *Cola anomala* nuts on plasma creatinine level.

Values are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; EACA200: hyperglycemic rats treated with 200 mg/kg.bw of C. anomala; EACA400: hyperglycemic rats treated with 400 mg/kg.bw of C. anomala; REF: Hyperglycemic rats treated

with 5 mg/kg.bw of glibenclamide. The values assigned with different letters (a, b, c, d) are significantly different (P<0.05).

DISCUSSION

The present study aimed to investigate the potential protective effect of the aqueous extract of *Cola anomala* nuts against hyperglycemia and oxidative stress generated by this condition. Postprandial hyperglycemia and fasting hyperglycemia being the two components of hyperglycemia, we began by the evaluation of the effect of the extract on postprandial hyperglycemia. After 30 min, a peak of hyperglycemia induced by the oral administration of a glucose solution was noted (Table 1). The extract not only limited the glycemic peak, but also resulted in a significant reduction in blood glucose level throughout the experiment, particularly at the 400 mg/kg.bw dose. The results thus obtained suggest a preventive effect of the aqueous extract of *Cola anomala* nuts on postprandial hyperglycemia. The alkaloids and flavonoids present in the extract could be responsible for this antihyperglycemic activity. Indeed, Gaikwad *et al*.[21] showed that alkaloids are inhibitors of intestinal carbohydrate digestion via their ability to bind to the active site of glycosidases, thus preventing the binding of their substrate. Similarly, flavonoids could block glucose transporters at the intestinal level, thus preventing intestinal glucose absorption[21]. Alkaloids also are the inhibitors of Dipeptidyl Peptidase-4 (DPP-4) promoting the availability of incretins and consequently increasing insulin secretion[22].

In order to evaluate the effect of the extract on the second component of hyperglycemia which is fasting hyperglycemia, an animal model of hyperglycemia was used. The injection of streptozotocin in normoglycemic rats led to a considerable increase in blood glucose after 24 hours, thus justifying the installation of hyperglycemia (Table 2). Aqueous extract of *C. anomala* nuts at 400 mg/kg.bw resulted in a significant decrease in blood glucose levels compared to untreated hyperglycemic rats (Table 2). This could be explained by the fact that the polyphenols present in the extract would have inhibited glucose 6-phosphatase and hepatic glucose 1,6 phosphatase which are the enzymes involved in gluconeogenesis as demonstrated by Bahadoran *et al*.[23].

Chronic hyperglycemia is at the origin of the development of oxidative stress, which is characterized by an imbalance between the antioxidant and prooxidant systems at the expense of antioxidants. This phenomenon could explain the decrease in antioxidant enzymes activities and the significant increase in pro-oxidant markers (NO and MDA) in plasma, liver and kidney as observed in untreated hyperglycemic rats compared to normal rats (Figures 1, 2, 3, 4). The extract improved endogenous antioxidant status by increasing SOD and renal catalase activity

(Figures 1 and 2) while decreasing plasma, liver and kidney MDA (Figure 3) and liver NO levels (Figure 4). This could be due to the antiradical activity of the polyphenols of the aqueous extract of *Cola anomala* nuts as demonstrated by Mbong *et al.*[10] thus preventing the inhibition of SOD and catalase[24]. In addition, the increase in antioxidant enzyme activity as observed at the renal level could also account for the ability of the polyphenols present in the extract to stimulate the expression of the nuclear factor NF-E2-related factor 2 (Nrf2). Indeed, Qader *et al.*[25] showed that some phytochemicals, in particular phenolic compounds (quercetin, curcumin...), act as natural activators of Nrf2, a transcription factor activated following oxidative stress. It induces the expression of its target genes and is one of the most important cellular defense mechanisms against oxidative stress[25].

The evaluation of the hepatic status of the animals (hepatoprotective or hepatotoxic) upon administration of STZ and the extract was done via the determination of ALT and AST activity. Altered activity of these biomarkers, as shown in the untreated hyperglycemic group (Figure 5), means that the liver, including the biliary system was negatively affected by administration of STZ. The elevated levels of these enzymes could be the result of inflammation of the liver cells, that led to a leakage of the enzymes into the bloodstream thus increasing their plasmatic levels[26]. In rats treated with the extract at the dose 400 mg/kg.bw, there was a significant decrease in AST activity, which could justify the non-toxicity as well as the hepatoprotective nature of the aqueous extract of *C. anomala* nuts (Figure 5) as demonstrated by Pradeepa *et al.*[27] with the decrease in AST, ALT and ALP (alkaline phosphatase) activities in diabetic rats treated with *Pithecellobium dulce* fruit extract.

Regarding renal function, it has been reported that about 15% of type 2 diabetics develop renal failure after 10 to 25 years of progression[28]. The excess of creatinine in the blood of untreated hyperglycemic rats compared to normal rats (Figure 6) may indicate glucotoxicity-induced renal damage. Ng *et al.*[29] showed that under diabetic conditions, there was renal hypertrophy accompanied by impaired glomerular filtration. Thus, the significant decrease in creatinine level in rats treated with aqueous extract of *Cola anomala at* the dose of 400 mg /kg.bw (Figure 6) proves an improvement in renal function through glomerular filtration. Chen and Zhang[30] justified the protective effect of organs by phenolic compounds contained in extracts of plants through, their role as antioxidants limiting the effects of pro-oxidants.

CONCLUSION

Aqueous extract of *Cola anomala* nuts reduces postprandial hyperglycemia and fasting hyperglycemia. It enhances the antioxidant status by increasing SOD and catalase activity while

decreasing Malondyaldehyde (MDA) and nitric oxide (NO) levels in rats subjected to hyperglycemia by STZ injection. Improving the biological levels of these different parameters could help limit diabetes-related complications affecting cellular tissues.

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