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RESEARCH ARTICLE

Effect of *Abengourou forastero cocoa* against doxorubicin-induced cardiotoxicity in experimental rats

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Abstract

Oxidative stress is a determining factor in the pathophysiology of heart disease. This study aimed at evaluating the protective activity of *Abengourou Forastero cocoa* in doxorubicin-induced cardiotoxicity in rats. Thirty (30) wistar rats divided into 5 groups were orally pretreated with distilled water, resveratrol (25 mg/kg/day) or defatted *Abengourou Forastero cocoa* powder (1000 and 1500 mg/kg/day) for sixty (60) consecutive days. A single dose of doxorubicin (15 mg/kg/day) was administered on day 59 by intraperitoneal route. The biochemical parameters were assessed and a histological examination of the heart was performed. This study showed that doxorubicin treated animals exhibited a significant increase of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, phosphocreatine kinases, creatine kinases, total cholesterol, triglycerides, and LDL-cholesterol levels in serum. However, a decrease of HDL-cholesterol and an alteration of cardiac tissue were noticed. Preventive treatment with *Abengourou Forastero cocoa* at doses of 1000 and 1500 mg/kg significantly reduced the biochemical and histological alterations induced by doxorubicin. Results showed that *Abengourou Forastero cocoa* protects and prevents against doxorubicin-induced cardiac damage.

Keywords: Forastero cocoa; Abengourou; doxorubicin; cardiotoxicity

Introduction

The heart is a vital organ of the human body, and despite all natural protective systems, is particularly vulnerable to toxic substances that can damage and disrupt its functions [1, 2]. The prevalence of heart disease remains very high in Africa, due to some risk factors such as smoking, alcoholism, physical inactivity, malnutrition, self-medication and environmental conditions [3, 4].

Therefore, toxins, drugs and hyperlipidemic diets play a key role by increasing oxidative stress [5, 6]. Cells or tissues sometimes suffer aggressions when they are subjected to endogenous or exogenous production of oxygen free radicals that go beyond their antioxidant capacities [7]. Our body has developed mechanisms to eliminate these free radicals. Thus, superoxide dismutases (SOD) are able to remove the superoxide anion by the dismutation reaction, forming, with two peroxides, an oxygen and a hydrogen peroxide molecule. As for reduced glutathione, it protects against oxygen radicals and peroxides or nitrogen monoxide (NO-). However, regarding the excessive release of free radicals that disrupt our antioxidant defense system, dietary supplementation with antioxidants such as polyphenols is strongly recommended [8].

In recent years, there has been a growing interest in plants with high polyphenol contents. This study highlighted the use of

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several natural or synthetic compounds with antioxidant properties to prevent and treat oxidative stress induced diseases [9, 10]**.** Among them, cocoa ranks high [11, 12].

Indeed, cocoa is an important source of polyphenols and account for 12 to 18% of its total dry weight, these are mainly flavonoids and phenolic acids. Its flavonoid content is much higher than that of green tea and red wine leaves [13]. The studies of [14] showed that *Abengourou Forastero cocoa*, a city in eastern Côte d'Ivoire, has a high polyphenol content with high antioxidant activity. According to these outstanding characteristics, the aim of this study is to investigate the effects of *Abengourou Forastero cocoa* intake against doxorubicin-induced damage in the heart of experimental rats. Thus, the biochemical parameters were assessed and a histological examination of the heart was performed.

Material and methods

Preparation of cocoa powder samples

Selected cocoa pods were harvested using a large knife and then split open with a club. Collected Cocoa beans were collected and then fermented at the edge of plantation for five days on a black tarpaulin and covered with banana leaves. After fermentation, cocoa beans were spread in the sun on a black tarpaulin for drying from 7 am to 4 pm for ten days. Beans were then peeled, crushed and manually degerminated using a knife. Cotyledons were ground with an electric mill (VIKING GE 150, Japan) to obtain cocoa mass which was pressed in a white cloth using a traditional press machine to extract cocoa butter. Finally, the press cake was ground in a mortar to give a fine cocoa powder used for pharmacological studies.

Experimental animals

Albino rats of Wistar strain (*Rattus norvegicus*) were used as experimental animals. Rats were raised at the animal house of the teacher training school in Abidjan (Côte d'Ivoire). They were between 2 and 3 months of age and weighing 150 -180 g. Animal were group-housed with 3 rats per plastic cage and kept at room temperature (29 $\pm$ 1°C) on a 12 h light/dark cycle. Rats had free access to water and food (food pellets and dry corn). The experiments were approved by the Ethical Committee of Health Sciences of University Félix Houphouët-Boigny (Côte d'Ivoire). These guidelines were in accordance with the European Council Legislation 86/609/EEC for the protection of experimental animals [15].

Study design

Animals were randomly divided into five (5) groups of six (6) rats:

- NORM Group (Normal control): received distilled water (1 mL/100 g body weight) by oral route for 60 consecutive days. On day 59, a solution of NaCl 0.9 % (0.5 mL/100g body weight) was intraperitoneally injected to rats.
- DOXO Group (intoxicated control): received distilled water (1 mL/100 g body weight) by oral route for 60 consecutive days. On day 59, doxorubicin (0.5 mL/100g body weight) was intraperitoneally injected to rats.
- REF Group (resveratrol, 25 mg/kg body weight). Received by oral route 1 mL / 100 g body weight of resveratrol (2.5 mg/ mL) for 60 consecutive days. On day 59, doxorubicin (0.5 mL/100g body weight) was injected to rats by intraperitoneal route.
- Group C1 (1000 mg/kg body weight of cocoa). Received by oral route 1 mL / 100 g body weight of cocoa (0.1 g/ mL) for 60 consecutive days. On day 59, doxorubicin (0.5 mL/100g body weight) was injected to rats by intraperitoneal route.
- Group C1.5 (1500 mg/kg body weight of cacao). Received by oral route 1 mL / 100 g body weight of cocoa (0.15 g/ mL) for 60 consecutive days. On day 59, doxorubicin (0.5 mL/100g body weight) was injected to rats by intraperitoneal route.

On completion of the experiment, the animals were anesthetized 24h later using diethyl ether and blood sample was collected from the carotid artery. Two (2) ml of blood was collected in a dry tube and centrifuged at 3000 rpm for 10 min and serum was collected to assess biochemical parameters. Then, each animal heart was collected, weighed, rinsed with distilled water and preserved in 10% formalin for histopathological studies.

The relative heart weight of each animal was evaluated as followed:

$$RHW = HW/BWD_{61}$$

- RHW : Relative Heart Weight
- HW : Heart Weight
- BWD61: Body weight of rat on day 61.

Biochemical analyses

Biochemical parameters such as Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), phosphocreatine kinases (CPK), creatine kinases, total cholesterol, triglycerides (TG), LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) were investigated by an automated analyzer (Cobas C311 HITACHI ROCHE, France) using a commercial kits according to the manufacturer's instructions.

Histopathological assay

Techniques used by Coujard et al [16] were adopted for this study. Samples preserved in aqueous formalin were successively dehydrated in five successive ethanol baths for 15 minutes each. The first two baths had respective concentrations of 70% and 90%. The other three contained absolute ethanol 96% pure. Tissues were embedded in toluene. The impregnation was carried out in an oven at 60°C in two successive paraffin wax for one hour each. The inclusion was performed in paraffin after solidification. Sections of tissues were cut at 5μm thickness using a microtome (Microm) and were stained with hematoxylin-eosin (H&E) solution. Finally, stained sections were examined under a light microscope Zeiss with an incorporated camera (Sony).

Data analysis

Data were analyzed using the software Graph Pad Prism 5.0 (Microsoft, USA). Comparison of means were performed with one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Statistical significance was considered at $p < 0.05$.

Results

Effect of treatments on cardiac enzyme parameters

As shown in table 1 serum enzyme activities of ALAT, ASAT, LDH, CPK and CK-MB of DOXO-group (intoxicated control group) were significantly higher ($p < 0.001$) compared to NORM group (normal control group). However, administration of cocoa powder or resveratrol to groups of intoxicated and treated animals (C1, C1.5 or REF) resulted in significantly lower level ($p < 0.001$) of ALAT, ASAT, LDH, CPK and CK-MB activity in serum compared with DOXO group (intoxicated control group). Thus, these activities did not reach those of normal control group.

Effect of treatments on serum lipid profile

Results tabulated in Table 2 indicated a significant increase ($p < 0.001$) level in total cholesterol, triglycerides and LDL-cholesterol and however an obvious significant decrease ($p < 0.001$) in HDL-cholesterol level in DOXO group compared with normal control group was observed.

These results also showed that treatment with cocoa powder at doses of 1 g/kg Pc (C1) or 1.5 g/kg Pc (C1.5) or resveratrol at a dose of 25 mg/kg Pc (REF group) promoted a significant decrease ($p < 0.001$) in total cholesterol, triglycerides and LDL-cholesterol level and an increase in HDL- cholesterol level compared with the DOXO group.

Effects of different treatments on the relative heart weight of rats

Results in Figure 1 indicated that the relative heart weight of normal control group ($0.31 \pm 0.02\%$) was significantly lower ($p < 0.001$) compared with DOXO group ($0.45 \pm 0.03\%$). Treatments with cocoa powder at doses of 1 g / kg body weight(C1) or 1.5 g / kg body weight (C1.5) or resveratrol at a dose of 25 mg / kg of body weight (REF group) caused a significant ($p < 0.001$) decrease in relative heart weight compared with DOXO group.

Effects of different treatments on rats heart structure

The histology of rats' heart tissues in the normal control group (NORM group) showed normal morphological aspects while the Doxorubicin treated group (DOXO group) exhibited morphological abnormalities such as severe myocardial necrosis with loss of myofibrils. Histology of heart tissues in resveratrol treated group (REF group) or cocoa powder treated groups at doses of 1 g / kg body weight (group C1) or 1.5 g / kg body weight (group C1.5) showed a decrease in Doxorubicin effects.

Discussion

Doxorubicin is an efficient and widely used chemotherapeutic agent. However, its clinical use is limited on account of its dose-dependent cardiotoxicity [17]. Experimental studies have proved that doxorubicin induces oxidative stress by releasing free radicals in myocardial cell [18]. Reactive oxygen species such as superoxide and hydroxyl radicals are likely to cause damage to various intracellular components. The myocardial tissue is particularly sensitive to free radical damage because it contains low levels of radical detoxifying enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH) and catalase (CAT).

Moreover, doxorubicin has high affinity for mitochondrial membrane phospholipids in cardiac myocytes, leading to doxorubicin accumulation in myocardial cell [19]. Doxorubicin accumulation in mitochondrial is hazardous for the heart since it could provoke extreme adverse effects on cardiac myocytes contractile function by altering energy metabolism [20].

Thus, doxorubicin-induced myocardial cell injury in rats is indicated by a high level of serum enzyme markers such as lactase dehydrogenase (LDH), phosphocreatine kinase (CPK) and transaminases ([21]. Increasing LDH levels in serum and extracellular fluid suggest a leakage of this enzyme from mitochondria as a result of doxorubicin-induced toxicity in animals. The above mentioned biochemical parameters were used as markers in other studies to assess cardiotoxicity [22].

Table 1 Effect of cocoaintake on cardiac enzyme parameters

Enzymatic parameters	Groups NORM	DOXO	REF	C1	C1.5
ALAT (UI/L)	82.2±3.3	169.8±7.3***	103.2±5.3***+++	119.5±2.4***+++	113.7±5.2***+++
ASAT (UI/L)	114.2±4.7	190.3±5.1***	133.0±3.3***+++	144.5±3.0***+++	139.2±3.2***+++
LDH (UI/L)	2187±38	3118±52***	2301±23***+++	2525±30***+++	2495±17***+++
CPK (UI/L)	2016±26	3107±27***	2105±14***+++	2300±16***+++	2228±21***+++
CK-MB (UI/L)	932±18	1378±24***	1005±17***+++	1098±26***+++	1056±13***+++

Data are presented as the mean ± SD (standard error) n= 6.* P < 0.05; ** P < 0.01; *** P < 0.001: significant difference compared with NORM group. + P < 0.05; ++ P < 0.01; +++ P < 0.001: significant difference compared with DOXO group.

Table 2 Effect of cocoa powder intake on serum lipid profile

lipid parameters	Groups NORM	DOXO	REF	C1	C1.5
C-T (UI/L)	0.62±0.02	1.04±0.02***	0.79±0.03***+++	0.80±0.02***+++	0.78±0.02***+++
TG (UI/L)	05.3±00.1	0.69±0.02***	06.0±0.01***+++	0.60±0.02***+++	05.9±0.02***+++
HDL-C (UI/L)	02.5±0.02	0.13±00.1***	0.23±0.02+++	0.23±0.01+++	0.22±0.02*+++
LDL-C (UI/L)	0.23±0.02	0.69±0.02***	0.40±0.02***+++	0.42±0.01***+++	0.41±0.02***+++

Data are presented as the mean ± SD (standard error) n= 6. * P < 0.05; ** P < 0.01; *** P < 0.001: significant difference compared with NORM group. + P < 0.05; ++ P < 0.01; +++ P < 0.001: significant difference compared with DOXO group.

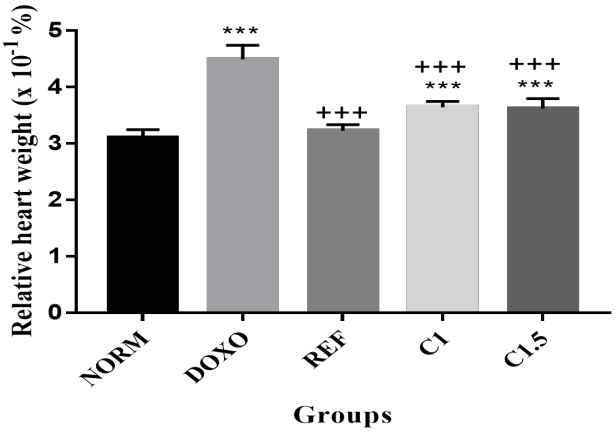

Figure 1 Effects of cocoa powder and resveratrol on relative heart weight. Data are presented as the mean ±SD (standard error) n= 6. * P < 0.05; ** P < 0.01; *** P < 0,001:significant difference compared with NORM group. + P < 0.05; ++P < 0.01; +++ P < 0.001: significant difference comparedwith DOXO group.
NORM group: distilled water +NaCl, DOXO group: distilled water +doxorubicin (15 mg/ Kg), REF group:resveratrol (25 mg / Kg) + doxorubicin (15 mg / Kg), group C1: cocoapowder (1 g / Kg) + doxorubicin (15 mg/ Kg), group C1.5: cocoa powder (1.5g / Kg) + doxorubicin (15 mg/ Kg).

Abengourou Forastero cocoa powder or resveratrol inhibits the release of CPK, LDH and transaminases induced by doxorubicin in rats' serum. It is widely reported that the production of free radicals induced by doxorubicin triggers off membrane peroxidation and disruption of cardiac myocytes, promoting a large release of CPK level in serum. However, Abengourou Forastero cocoa powder administered to animals inhibit the release of CPK, LDH and transaminases resulting in an outstanding restoration of serum enzyme activities. These results are in agreement with those of Murat et al. [23].

In addition, increase levels of plasma triglycerides, total cholesterol, and low density lipoprotein (LDL) in doxorubicin treated rat show that this antibiotic may interfere with lipid metabolism or biosynthesis. Pretreatment with cocoa powder or resveratrol decreased blood lipid level with an increase in high density lipoprotein (HDL) level. Indeed, the decrease in blood lipids and the increase in HDL-cholesterol in cocoa

treated groups of animals may be due to polyphenols. The lipid-lowering effect of cocoa is due to the inhibition of hepatic cholesterol biosynthesis, to an increase secretion of fecal bile acids and stimulation of LDL-cholesterol catabolism receptors and to an increase uptake of LDL-cholesterol in blood by the liver [24]. This lipid profile improvement could better heart activity.

Improvement of lipid parameters could be due to cocoa butter, composed of stearic acid (35%), palmitic acid (25%), oleic acid (35%) and linoleic acid (2%). Cocoa butter therefore, contains 60% of saturated fatty acid [11]. Several studies showed a correlation between diets rich in saturated fatty acid and an increase in total cholesterol. The current nutrition trend is to minimize the intake of saturated fatty acids that increase blood LDL-cholesterol, a risk factor for cardiovascular disease. However, the "atherogenic power" of saturated fatty acids depends on chain length and the body's ability to metabolize them [25].

While palmitic acid enhances production of LDL-cholesterol, stearic acid, which is predominant in cocoa, is considered non-atherogenic. Indeed, according to Chaveron [26] and Keen et al. [11], once in the intestine, it is metabolized to oleic acid. Thus, oleic acid, its precursor and linoleic acid (polyunsaturated acid) in cocoa butter could promote an uptake of cholesterol to the liver for its elimination. This elimination could help reducing total cholesterol level in the body for a better cardiac activity [27].

What is more, the histopathological assay revealed that *Abengourou Forastero cocoa* powder alleviate alteration of heart tissue in cocoa powder treated groups of rats (Group C1 and Group C1.5) compared with DOXO group. These results confirm the ability of *Abengourou Forastero cocoa* to reduce toxic effects of doxorubicin by restoring normal cardiac physiology disrupted by this cardiotoxic substance (doxorubicin).

Conclusion

The results of this study revealed that *Abengourou Forastero cocoa* powder alleviate doxorubicin-induced cardiac cell damage. Therefore the use of this cocoa is strongly recommended to protect and prevent against heart damage. This protective activity was exhibited by an improvement in heart biochemical parameters and by a significant decrease in relative heart weight. This protective activity was significant at a dose of 1 g / kg body weight and its mechanism could be based on their antioxidant and antiradical activities due to phenolic compounds.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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