

Antidiabetic and Antiplatelet Aggregation Activities of *Bridelia ndellensis* Stem Bark Extracts

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Abstract This study was undertaken to investigate the antidiabetic and antiplatelet activities of *Bridelia ndellensis* ethanol extract and its fractions in type 2 model diabetic rats. After 28 days repeated oral feeding, the ethanol extract (50 mg/kg) significantly ($p < 0.05$) reduced the levels of blood glucose, total cholesterol and triglycerides levels in NIDDM rats. ADP-induced platelet aggregation *in vitro* was also inhibited by the parent ethanol extract in a concentration-dependent manner. All concentrations (4, 8, 12 and 16 mg/ml) of the extract produced significant inhibitory effects, the more pronounced effect (93.31% inhibition) was observed with the highest concentration (16 mg/ml). Ethyl acetate, butanol and water fractions from the 80% ethanol extract of *B. ndellensis* also significantly ($p < 0.001$) inhibit platelet aggregation. The water part is the most active extract with an inhibitory effect of 85.97%. These results suggest that *B. ndellensis* ethanol extract possesses antidiabetic effects in type 2 model diabetic rats. This plant extract and its fractions contain active principles against platelet aggregation and could therefore be beneficial for the prevention of cardiovascular diseases related to diabetes.

Keywords Diabetes, Platelet Aggregation, *Bridelia ndellensis*, Type 2 Diabetic Rats

1. Introduction

Diabetes is a serious chronic metabolic disorder that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system[1]. In 2007, there were approximately 171 million diabetic patients worldwide and this figure is likely to be more than double by 2030[2]. Existing therapeutic agents for Diabetes Mellitus suffer from considerable limitations such as hypoglycemic risk, cardiovascular complications and overweight[3,4]. Management of diabetes and the metabolic syndrome without any side effects is still a challenge to the medical system. This leads to increasing search for improved antidiabetic drugs since approach of scientists for synthesis of these drugs has not yet brought expected results[5]. It is well recognized that bio-resources such as plants, microorganisms and animals have tremendous potential in providing bioactive agents for pharmaceutical purpose[6]. WHO estimates that about 80% of the people in developing countries rely on medicinal plants for their primary health needs[7]. Few of the plant treatments used traditionally for

diabetes have received scientific scrutiny[6,8], and the World Health Organization (WHO) recommended accordingly that this area warrant more attention[7].

Bridelia ndellensis Beille is a plant belonging to Euphorbiaceae family. This medicinal plant is commonly used in Cameroon against fever, rheumatism, diarrhoea, and diabetes. *B. ferruginea*, another specie from the same genus, is well known for its hypoglycemic activity[9,10]. Recently, we reported the acute antihyperglycemic effects of *B. ndellensis* ethanol extract in streptozotocin-induced type 1 model rats[6].

This study was therefore undertaken to evaluate the antidiabetic and antiplatelet activities of *B. ndellensis* ethanol extract and its fractions in type 2 diabetic model rats in a chronic experiment.

2. Materials and Methods

2.1. Collection of plant Material

B. ndellensis stem bark was collected from Ngaoundere, Adamawa Region, Cameroon. Botanical identification was performed at the national herbarium, Yaounde, Cameroon and herbarium voucher specimen number 9676/HNC has been deposited. Bark samples were dried under sunlight and powdered.

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2.2. Extraction Procedure and Fractionation

A 2.5 kg powdered *B. ndellensis* bark was extracted (4 times, 24 hours each time) with 80% ethanol at room temperature, filtered and concentrated *in vacuo* (40 °C) and freeze-dried to obtain a 200 g extract (8%w/w) using a Varian 801-model LY-3-TT freeze-dryer (USA). The dried extract was suspended in water (500 ml) and then partitioned successively with CH₂Cl₂, EtOAc and 1-BuOH to give 12.92 g, 20.60 g and 30.82 g of extracts, respectively. The parent extract (80% EtOH) and its dichloromethane (DCM), ethyl acetate (EA), 1-butanol (BU) soluble parts were tested on NIDDM model rats.

2.3. Animals

Adult male Wistar rats weighing 180 - 220 g were used throughout the study. The animals were bred and maintained on a 12 h light-dark cycle. Water and a standard laboratory pellet diet were supplied *ad libitum*. Experiments were carried out in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee.

2.4. Induction of Type 2 Diabetes

Type 2 diabetes (Non-Insulin Dependent Diabetes Mellitus, NIDDM) was induced as previously described by Bonner-Weir[11]. In brief, Streptozotocin was freshly prepared in citrate buffer (0.1 M, pH 4.5) and injected at a dose of 90 mg/kg bw to 48 h old pups. Experiments were carried out 3 months later when their body weight was approximately 175 - 180 g. Prior to the experiments, the diabetic status of the rats was checked by an oral glucose tolerance test (OGTT) because, it has been shown that the fasting blood glucose level is almost normal in 2 days old streptozotocin-induced type 2 diabetic rats model[12]. OGTT was performed by given 2.5 g/kg of glucose to rats fasting for 12 h prior to the test and the serum glucose was checked before and 30 min after glucose load. Rats with 30-min serum glucose level more than 10 mmol/l were considered as diabetic and separated into groups of 6 to 8 rats for trials with plant extracts and standard drugs.

2.5. Experimental Procedure

Two groups of type 2 diabetic rats (6 rats each) were fed plant extract at doses of 25 and 50 mg/kg bw, twice daily for 28 consecutive days. Another two groups of nondiabetic and diabetic rats received distilled water (10ml/kg, bw) and were considered as normal and diabetic controls respectively. Food and water were provided *ad libitum* throughout the experimental period. Body weight of each rat was recorded weekly. At the end of the experiment, the animals were deprived of food overnight and blood samples collected by cardiac puncture under mild ether anesthesia. Serum was then prepared by centrifugation (3000xrpm, 10 min) for the analysis of glucose, total cholesterol, HDL-cholesterol and triglycerides.

2.6. Biochemical Analyses

Serum glucose level was determined by the glucose oxidase (GOD-PAP) based enzymatic method using kits from Boehringer Mannheim GmbH (Germany). Serum total cholesterol, HDL-cholesterol and triglycerides levels were determined by enzymatic colorimetric using commercial kits from SERA PAK, USA.

2.7. Effects of *B. ndellensis* Ethanol Extract and its Fractions on Platelet Aggregation

To evaluate the beneficial effect of the treatment with plant extracts on some diabetes complications, platelet aggregation was evaluated. Platelet aggregation test was performed on platelets from rat plasma (platelet rich plasma and platelet poor plasma).

2.7.1. Preparation of Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP)

Rats were anesthetized with sodium pentobarbital (50 mg/kg, bw) and blood samples were immediately collected from the abdominal aorta in a 3.8% sodium citrate solution. Samples were carefully centrifuged at 800 rpm for 15 min at 20°C. The supernatant (PRP) was then transferred into a polypropylene plastic tubes for the estimation of platelet aggregation. After the preparation of PRP, the remaining blood samples were again centrifuged at 3000 rpm (1000 g) for 20 min at 20°C. The supernatant namely platelet poor plasma (PPP) was then transferred into polypropylene plastic tubes and used for the estimation of platelet aggregation.

2.7.2. Evaluation of Platelet Aggregation

Platelet aggregation test was performed as previously described by[13]. Before the test, the aggregometer (Chrono Log Lumi aggregometer, Chronolog Corp. Havertown, PA, USA) was prewarmed for 20 min. Aliquots (500 µl) of the platelet suspension (PRP) were first incubated for 5 min with 40 µl of plant extracts or vehicle alone in the cuvette of a at 37°C with constant stirring (1000 rpm). Extracts solutions used were concentrated at 4, 8, 12 and 16 mg/ml for the 80% ethanol extract and 16 mg/ml for the ethyl acetate, butanolic and water fractions. Subsequently, 6µl of the platelet stimulating agent (ADP) was added. Maximal aggregation response was defined as the maximum change in light transmission observed over 5 min in the presence of vehicle without extract, and anti-aggregant activity was defined as the percentage reduction of this response in the presence of the extract.

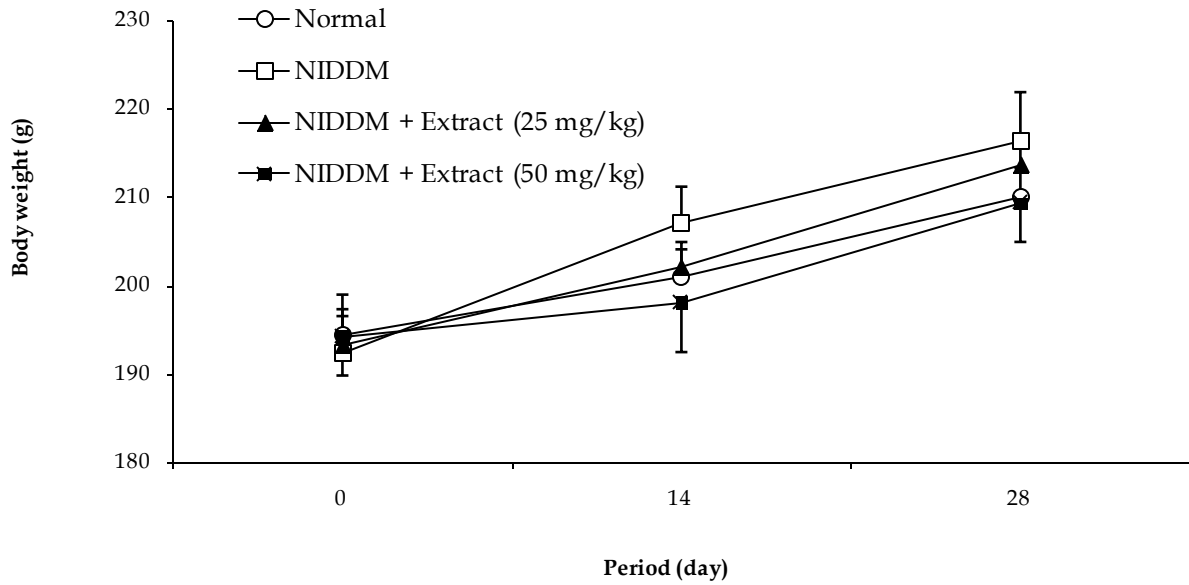
3. Results

3.1. Effects of 80% Ethanol Extract of *B. ndellensis* on Body Weight and Serum Glucose Levels

The body weights in normal and NIDDM rats were increased from the beginning (day 0) to the end of the

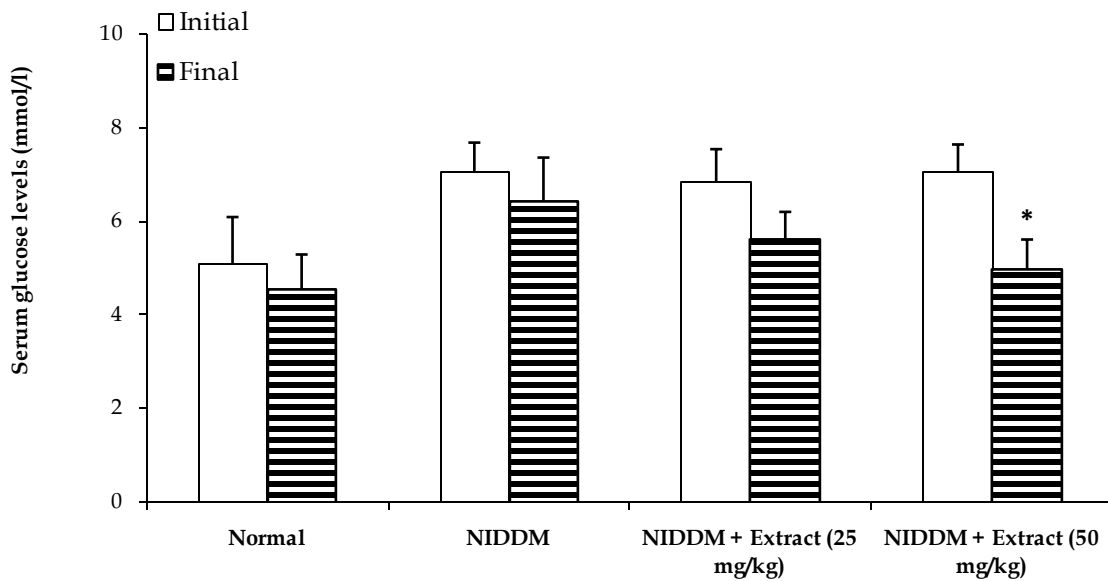
experiment (day 28 day). In diabetic treated rats, the increases were less important compared to non-treated normal and diabetic rats. Differences observed among groups were not significant (Figure 1). As shown in figure 2, a daily administration of 80% ethanol extract of *B. ndellensis*

for 28 days in NIDDM rats decreased their blood glucose levels when compared with the respective initial values. This reduction was more pronounced (29.60%) and significant ($p < 0.05$) for the dose of 50 mg/kg.



Values are mean \pm S.E.M., n=6

Figure 1. Effects of 80% ethanol extract of *B. ndellensis* on body weight of experimental animals after 28 days of treatment



Values are mean \pm S.E.M., n=6. * $p < 0.05$ compared to the initial value

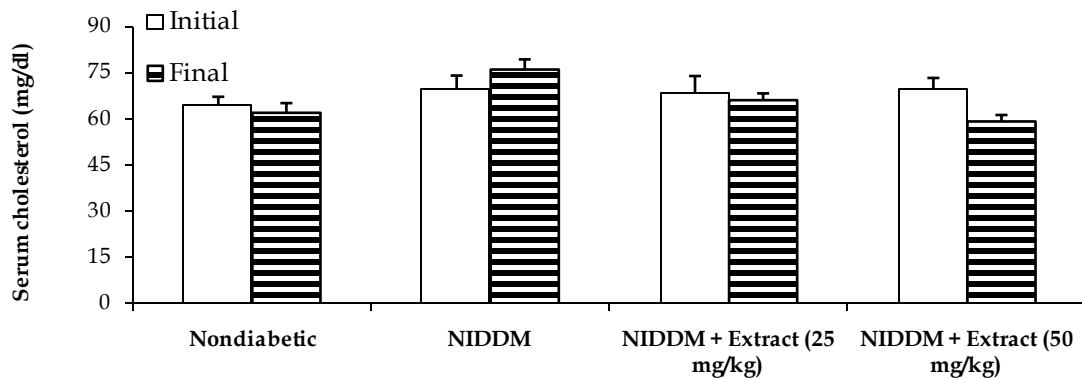
Figure 2. Effects of 80% ethanol extract of *B. ndellensis* on serum glucose levels of experimental animals after 28 days of treatment

3.2. Lipid Profile of NIDDM Rats after 28 Days of Treatment with the Ethanol Extract of *B. ndellensis*

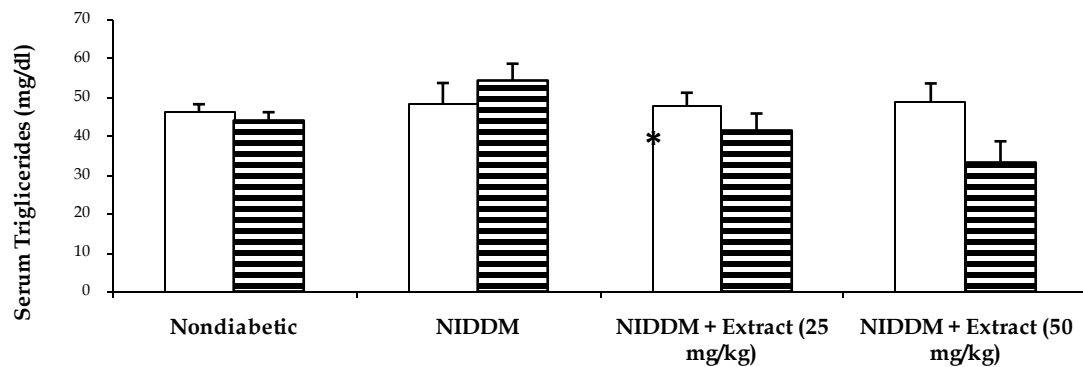
Figure 3 shows the serum levels of total cholesterol, triglycerides, HDL cholesterol of normal and experimental diabetic animals. Total cholesterol and triglycerides were significantly increased in diabetic animals compared with normal. However, rats treated with *B. ndellensis* extract at the dose of 50 mg/kg showed a significant ($p < 0.05$) decrease in total cholesterol and triglycerides levels at the end of the experiment as compared with their respective initial levels. At the dose of 25 mg/kg, the decrease was significant ($p < 0.05$) only for triglycerides. HDL-cholesterol levels were decreased in NIDDM control rats compared with normal control. However, there is no significant increase of HDL-cholesterol levels in NIDDM rats treated with *B. ndellensis* extract at doses of 25 and 50 mg/kg.

3.3. Effect of 80% Ethanol Extract and Fractions of *B. ndellensis* on on ADP-induced Platelet Aggregation

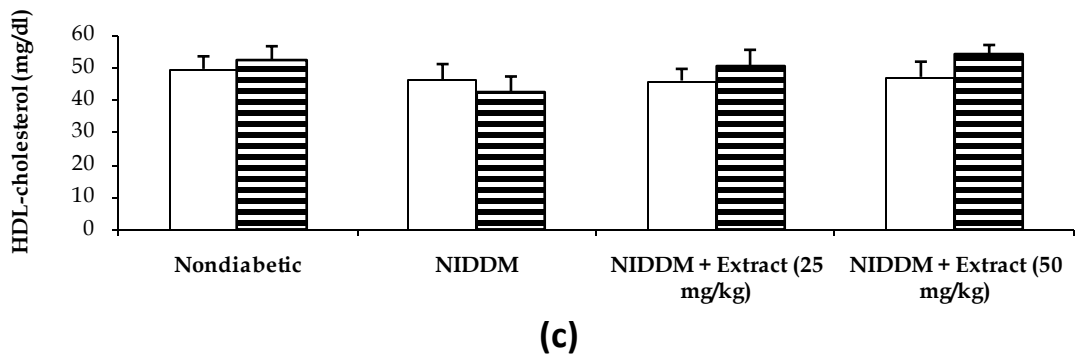
The effect of 80% ethanol extract of *B. ndellensis* on ADP-induced platelet aggregation in nondiabetic rat platelet rich plasma (PRP) *in vitro* is shown in figure 4. PRP from nondiabetic rats in the presence of ADP leads to 100% platelet aggregation. When PRP is incubated with 80% ethanol extract of *B. ndellensis* at different concentrations before induction of aggregation with ADP, a concentration-dependent inhibitory effect of the extract was observed. The extract at all concentrations caused significant ($p < 0.001$) inhibition of platelet aggregation induced by ADP. The inhibitory percentages observed with the extract were 29.96, 47.08, 73.35 and 93.31% respectively with the concentrations of 4, 8, 12 and 16 mg/ml. As the dose 16 mg/ml induced the highest inhibitory effect, this dose was used to evaluate the effect of the ethanol extract fractions on ADP-induced platelet aggregation. Ethyl acetate, butanol and water fractions from the 80% ethanol extract of *B. ndellensis* significantly ($p < 0.001$) inhibit platelet aggregation (Figure 5). The water part is the most active extract with an inhibitory effect of 85.97%.



(a)

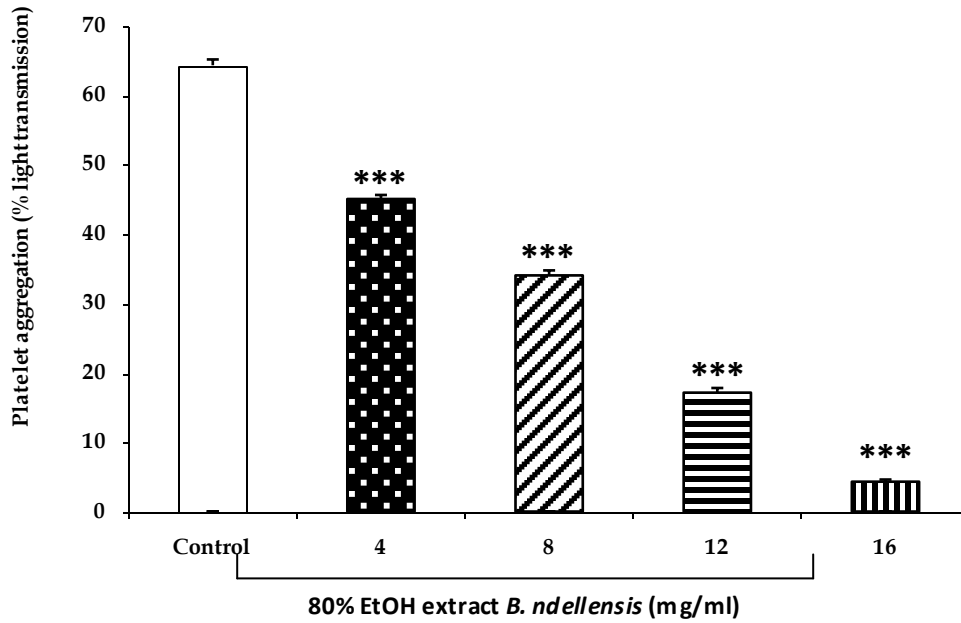


(b)



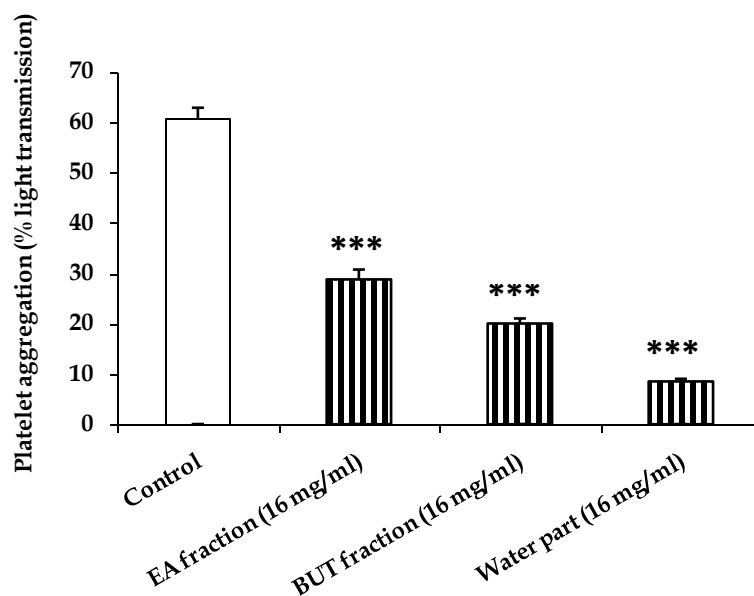
Values are expressed as mean ± S.E.M., n=6. *p<0.05 and **p<0.01 Compared with initial values.

Figure 3. Serum cholesterol (a), triglycerides (b) and HDL-cholesterol (c) levels in nondiabetic and NIDDM rats before and after 28 days oral treatment with the ethanol extract of *B. ndellensis*



Values are mean ± S.E.M., n=8. PRP: platelet rich plasma. ***p<0.001 compared to platelet poor plasma control

Figure 4. Effect 80% ethanol extract of *B. ndellensis* on ADP-induced platelet aggregation in nondiabetic rat PRP



Values are mean \pm S.E.M., n=8. PRP: platelet rich plasma. ***p<0.001 compared to platelet poor plasma control

Figure 5. Effect *Bridelia ndellensis* ethanol extract fractions on ADP-induced platelet aggregation in nondiabetic rat PRP

4. Discussion

In a 28-period chronic feeding experiment, the ethanol extract of *B. ndellensis* significantly reduced the blood glucose level and improved body weight in NIDDM rats. In addition to this chronic effect, we described in a previous study the antihyperglycaemic effect of the extract in an acute glucose-fed state and its insulinotropic effects[6]. Therefore, the activation of insulin receptors at peripheral target tissues by the extract became apparent. Insulin stimulates glucose uptake in muscle and adipose tissue by translocating intracellular glucose transporter[14]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. Hypertiglyceridemia is also associated in metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose intolerance[15]. In this study, a daily administration of *B. ndellensis* ethanol extract (50 mg/kg) to NIDDM rats twice a day for 28 consecutive days significantly reduced the levels of total cholesterol and triglycerides levels. The extract also increased the level of HDL-cholesterol although this increase was not significant compared to untreated-NIDDM rats. As the extract increased HDL-cholesterol and decreased total cholesterol, it thus has the potential to prevent the formation of atherosclerosis and coronary heart disease which are the secondary diabetic complications of severe diabetes mellitus. Increase in HDL cholesterol is associated with a decrease in coronary risk[16]. Hence the resulting benefit of the extract is not only to combat hyperglycemia, but also to prevent dyslipidemia and insulin resistance which are important risk factors for the micro- and macro-vascular complications of diabetes. On the other hand, the extract may act on lipid

profile by decreasing the serum non-esterified fatty acids (NEFA) in diabetic rats and thereby reducing the level of triglycerides. Similar results have been previously observed with masoprocol (nordihydroguaiaretic acid), a pure compound isolated from *Larrea tridentata*[17]. Several line of evidence indicate that the increased thromboxane production and thereby platelet aggregation which is an important marker of inflammation, may be related primarily to high concentration of glucose and lipids[18, 19]. In the present study, the ethanol extract of *B. ndellensis* and its fractions significantly inhibited ADP-induced platelet aggregation *in vitro*, the aqueous fraction being the most active (water part > butanol fraction > ethyl acetate fraction). This result indicates that *B. ndellensis* bark contains anti-platelet principles which are mostly polar. This inhibitory effect of the extract on platelet aggregation may be explained by the decrease observed in blood glucose and triglycerides levels of diabetic rats treated for 28 days. Enhanced platelet aggregation has been reported in type 2 diabetic patients who are characterized by glucose intolerance and dyslipidemia[20].

5. Conclusions

From this study, we can conclusively state that *B. ndellensis* stem bark ethanol extract has beneficial effects on blood glucose as well as improving hyperlipidemia due to diabetes. This plant extract contains anti-platelet principles which are mostly. *B. ndellensis* also contains hypolipidemic principles, and constitutes a good tool for the management of dyslipidemia and cardiovascular complications related to diabetes.

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