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Aqueous Extract of *Alstonia boonei* Bark Reduces Chronic Hyperglycemia and Prevents its Complications through Increase of Hepatic Global Dna Methylation in Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author MF designed the study project and supervised the work. Author WTA carried out the experiment. Authors RFN and GTN made statistical analysis, authors MF and WTA drafted the manuscript, author BAK read critically the manuscript. Authors LNJ and JOE supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: DNA methylation profile is involved in several physiological processes. Its alterations in the liver of diabetic patients characterized by global hypomethylation are associated with the pathophysiology of type 2 diabetes and its complications. The present study has evaluated the effect of the aqueous extract of *Alstonia boonei* barks on the global methylation of hepatic DNA in association with hyperglycemia and diabetes complications induced by high-fat diet (HFD) feeding

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and administering of streptozotocin (STZ) which mimics the metabolic abnormalities very similar to those seen in human Type 2 diabetes.
Methods: *A. boonei* barks were harvested, processed, dried, ground and an aqueous extraction was prepared (ratio 1/10 w/v). An *in vivo* study was conducted in an animal model of high-fat-streptozotocin (HF-STZ) induced diabetes. Rats were divided into five groups of five rats each: a normoglycemic group, an untreated hyperglycemic group, three hyperglycemic groups including two test groups receiving aqueous extract of *A. boonei* barks (AEAB) by esophageal gavage at the doses of 200 and 400 mg/kg body weight once daily and a reference group receiving metformin at 10 mg/kg body weight. After 28 days of experimentation during which fasting blood glucose levels were taken every 14 days under fasting conditions, the animals were sacrificed. Plasma and liver homogenate samples from the sacrificed rats were used for biochemical assays (markers of oxidative stress such as malondialdehyde level, superoxide dismutase (SOD) and catalase activity, and markers of lipid profile such as total cholesterol, and triglycerides, HDL-c, LDL-c and VLDL-c). The analysis of the global DNA methylation profile was performed by the immunoprecipitation. Pearson's correlation was used to evaluate the relationship between the values.
Results: The aqueous extract increased the hepatic DNA methylation by 0.41% and 0.63% at 200 and 400 mg/kg body weight, respectively, compared to metformin (0.47%±0, 05). This effect was significantly associated with the hypoglycemic effect obtained at 400 mg/kg body weight with a decrease in initial blood glucose level of -29.87%.
Conclusion: AEAB reduces chronic hyperglycemia and prevents its complications by increasing global hepatic DNA methylation.

Keywords: DNA methylation A. boonei bark extract; hyperglycemia; diabetes complications.

1. INTRODUCTION

Scientific and technological progress has transformed the world, with considerable changes in lifestyle, including unhealthy eating habits, thus increasingthe incidence of non-communicable diseases, including diabetes (Wells, 2006). Diabetes is characterized by chronic fasting hyperglycemia, due to a relative or absolute deficit of insulin secretion and/or action [1]. According to the International Diabetes Federation (IDF), more than 463 million people live with diabetes worldwide and a prediction of 700 million by 2045 if nothing is done [2]. Cameroon does not escape as it has 615,300 people with diabetes [3]. Data or evidences have reinforced the involvement of epigenetic mechanisms in the etiology of type 2 diabetes as well as the evolution of its complications [4]. Epigenetics defines the set of transmissible and reversible modifications of gene expression without changes in the DNA sequence (Monhonval et *al.,* 2014). These modifications involve DNA methylation, post-translational modifications of histones and modifications by non-coding RNAs. DNA methylation being the most studied epigenetic modification in the regulation of gene transcription and inactivation [5]. DNA methylation reactions take place mainly in the liver. They involve s-adenosylmethionine (SAM) as a methyl donor [6]. Alteration of DNA methylation in the liver has been associated with dysregulation of hepatic metabolism observed in diabetic patients altering regulation of fasting blood glucose and postprandial glucose [6]. Other evidences suggest that under hyperglycemic conditions, there is a disturbance in the metabolism of monocarbons in the liver, an overproduction of reactive oxygen species (ROS) which are responsible for a decrease in the SAM pool, a decrease in the activity of DNA methyltransferases and an increase of demethylation enzymes, resulting in an overall hypomethylation of DNA (Sarra et *al.*, 2015). Hepatic DNA hypomethylation has been associated with overexpression of the protein kinase C epsilon gene involved in hepatic insulin resistance [7]. Thus, prevention or correction of global hepatic DNA hypomethylation could have potential beneficial effects in the management of diabetes and its complications. Some epigenetic drug like Minocycline prevents active DNA demethylation and complications of hyperglycemia, by inhibiting poly(ADP-ribose) polymerase 1. On the other hand, synthetic compounds are often associated with side effects (gastrointestinal disorders, liver damage) [1]; and are of high cost for certain social classes. For this reason, the urgent search for natural compounds with low costs and side effects is necessary.

Medicinal plants are rich sources of bioactive compounds including alkaloids and phenolic compounds [8]. They are endowed with multiple biological activities among others antioxidant,

anti-cancer, cardio-protective, neuro-protective, hypoglycemic and anti-inflammatory activities [9,10]. Recent study has shown their beneficial effects on the inhibition of epigenetic alterations associated with cancers [11]. *Alstonia boonei,* a plant of the Apocynaceae family is widely used in many African countries.The phytochemical screening of *A. boonei* stembarks and leaves reveal the present ofPhenols; Flavonoids;Alkaloids; Tannins;Saponosides andTerpenoids [12]. This plant is known for its high reputationas an anti-malarial [13]. The decoctions of barks are used to clean wounds, fight insomnia and diarrhea [14]. It is also endowed with anti-microbial, hypotensive, hypoglycemic and anti-inflammatory activity, [15,16,17,13]. The objective of this study was to evaluate the effect of aqueous extract of *Alstoniaboonei* barks on the modulation of DNA methylation and to evaluate the link with the anti-diabetic property of the extract.

2. MATERIALS AND METHODS

2.1 Plant Material

Alstonia boonei barks were collected in May 2020 in Mbaligui locality (Central Region, Cameroon). The plant was identified at the National Herbarium of Cameroon under the voucher number *43368/HNC.* Barks were isolated, once at the Laboratory of Nutrition and Nutritional Biochemistry (LNNB). They were cleaned, dried at room temperature until constant weight, and then ground in a blender (Philips Stay Fresh HR3752/00).

2.2 Preparation of the Aqueous Extract of *A. boonei* Barks (AEAB)

Four hundred grammes (400 g) of powder of *A. boonei* were macerated in 4000 mL of distilled water (1:10 w/v ratio) for 24 hours at room temperature. The mixture was shaken at intervals to ensure thorough extraction. The macerated extract was filtered with Wattman paper number 3 (Whatman International Limited, Ken, England). The filtrate was evaporated in the oven (WGLL-65BE) at 50°C for 72h and the extract (AEAB) obtained was stored at 4°C for further use.

2.3 *In vivo* Experiment

2.3.1 Food composition

Table 1 shows the composition of the diets used in this study.

2.3.2 Animals

Twenty-five (25) male albinos *Wistar* rats weighing between 210 and 250 grams were provided by the animal house of the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of the Department of Biochemistry, Faculty of Science, University of *Yaoundé* I and were acclimated for 7 days before used. None of these animals had been subjected to previous experiments and showed no signs of abnormalities. The rats were placed in standard cages at room temperature and subjected to a 12-hour day-night cycle with good aeration and natural light. The bedding was made of sawdust and changed every two days to ensure good hygienic conditions and the animals had free access to water and were fed with a standard diet with an unlimited access to tap water daily. The experimental protocol and the maintenance of the laboratory animals were carried out under the standard ethical guidelines for the use of laboratory animals and care as described in the guidelines of the European Community and the Ethics Committee of the Faculty of Science of the University of Yaounde I.

2.4 Study Design

Diabetes was induced according to the protocol descript byUmashanker et al. [18] with some changes. Rats were randomly divided into two groups: a negative control (NC) group of 5 rats, fed with a normal diet, and the HF-STZ group made of 20 rats, fed with the high-fat diet. The animals received their respective diets for a period of 8-week, and at week-0, after mild ether anesthesia, a single dose of 50 mg/kg of streptozotocin (STZ) dissolved in citrate buffer (100 mM; pH 4.5; 150 mM NaCl)was administered intraperitoneally to animals fasted for 12 h in the high-fat-streptozotocin (HF-STZ) group. The control animals received only vehicle (100 mM citrate buffer, pH 4.5). One hour (1h) after STZ administration, the animals were given a glucose solution (20%) to avoid hypoglycemic shock. After twenty-four hours, the blood glucose level of the rats was assessed by the glucose oxidase method using test strips and a glucose meter (Gluco-Plus brand ^{TM}). Animals with blood glucose levels greater than or equal to 200 mg/dL were diagnosed as diabetic [19]; and allocated to different groups for further experimentation as follows:

- Group I (negative control rats): non-diabetic rats receiving distilled water by daily gavage;

- Group II (positive control): untreated diabetic rats receiving distilled water by daily gavage;
- Group III (test 1): diabetic rats receiving AEAB by daily gavage at a dose of 200mg/kg bw;
- Group IV (test 2): diabetic rats receiving AEAB by daily gavage at a dose of 400mg/kg bw;
- Group V (reference): diabetic rats receiving metformin by daily gavage at a dose of 10mg/kg bw.

The volume of administration was 5 mL/kg bw. During the experiment, fasting blood glucose levels were taken for each group at regular time intervals of seven (7) days during the 28 days of experiment. Fasting blood glucose was expressed as percentage of variation.

$$\% \text{ Blood glucose level} = \frac{\text{Glycemic(GX)} - \text{Glycemic (G0)}}{\text{Glycemic (G0)}} \%$$

G0: Blood glucose at day = 0, Gx: Blood glucose after each 7 days.

2.4.1 Euthanasia of animals and preparation of biological samples

At the end of the experiment (Day 28), the animals were fasted for 12 hours, and the blood glucose level of each rat was taken. Under mild chloroform anesthesia, they were euthanized by cervical decapitation. Blood was collected on EDTA tubes for subsequent DNA extraction and biochemical analysis. Similary, the liver was isolated by dissection and washed in NaCl solution (0.9%), wrung out, weighed, and then preserved in a previously prepared dimethylsulfoxide (DMSO) saline solution **(**DMSO 20%; Sodium-EDTA 0.25M; NaCl saturating, PH 7.5) for subsequent DNA extraction.

2.4.2 Extraction of DNA from blood and liver tissue samples

DNA extraction from blood was performed according to the protocol described by Plower et al. [20] based on the properties of chelex-100; while DNA from liver was isolated according to the protocol described by Coombs et al. [21]. To verify the reliability of the extractions, the DNA concentration was determined by spectrophotometry using the Nanodrop 2000 (Thermo scientific with Nano Drop software) and the purity assessed from the Abs260/Abs280 ratio. DNA extracts were diluted with triethyl buffer (1X; pH 7.5) to 100 ng/µL for liver DNA and 50 ng/µL for blood DNA.

2.4.3 Analysis of the global DNA methylation profile

Analysis of the global DNA methylation profile from the DNA samples was done using the methylated DNA immunoprecipitation method from the MethylFlash™ *Global DNA Methylation (5-mC) ELISA Easy* Kit (catalog: P-1030) according to the supplier's protocol. Briefly described for that, a volume of 100 µL of binding solution was added to all the wells, then 2 µL of negative control was added to the negative control wells, 2 µL of positive control at different concentrations (0,1; 0,2; 0,5; 1; 2;5 %) were added to the positive control wells to generate a standard curve, and 4 µL of DNA sample (100 ng) were added to the test wells. The mixture was then homogenized and incubated at room temperature for 60 minutes. After incubation, the binding solution was removed from each well and the wells were washed with 150 µL of diluted wash buffer three times. Subsequently, 50 µL of the 5 mC antibody complex solutions was added to the wells and the wells were incubated at room temperature for 50 minutes. After incubation, the detection complex solution was removed from each well and the wells were again washed with 150 µL of diluted wash buffer five times. A volume of 100 µL of the development solution was added to each well. The whole set was gently shaken against a flat surface for 10 seconds and incubated at room temperature for 4 minutes. The enzyme reaction was stopped by adding 100 µL of the stop solution to each well and the absorbance was read on a micro plate reader at 450 nm. The percentage of 5-methylcytosine was calculated from the following formula:

$$\%5\text{mC} = \left(\frac{(\text{Abs sample} - \text{Abs negatif control})}{\text{Slope x S}}\right) \text{x}100$$

Where S represents the amount of DNA in the sample in ng

2.4.4 Oxidative status of the liver

As a marker of hepatic oxidative stress, the level of malondialdehyde, an indicator of lipid peroxidation, was determined using Yagi [22] protocol.The activity of enzymes of the hepatic antioxidant system such as superoxide dismutase (SOD) was assessed using the method of Misra and Fridovish [23] and catalase activity throught the method of Sinha [24].

2.4.5 Plasma lipid profile

Total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were assessed in plasma through enzymatic methods using chronolab kit. Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were determined from the formula of Friedewald and et al., [25].

LDL - c (mg/dL) = TC - (HDL - c +TG/ 5)

VLDL - c (mg/dL) =TG/ 5

2.4.6 Atherogenic indices evaluation

The lipid profile parameters were used to evaluate the Atherogenic Index of Plasma (AIP), Atherogenic Coefficient (AC), Cardiac Risk Ratio (CRR) and Cardioprotective Index (CPI) according to the following formulas:

CRR1 $=\frac{\mathrm{TG}}{\mathrm{HDL-C}}$; CRR2 = $\frac{\mathrm{LDL-c}}{\mathrm{HDL-C}}$ [26];

AC = $\frac{\mathrm{TG-HDL-C}}{\mathrm{HDL-C}}$ [27];

AIP = $\log\frac{\mathrm{TG}}{\mathrm{HDL-C}}$ [28];

CPI =$\frac{\mathrm{HDL-C}}{\mathrm{LDL-C}}$ [29].

2.5 Statistical Analysis

The Microsoft Excel software was used to process data and plot the graphs. Statistical Package for Social Science *(SPSS*) version 20.0 for Windows was used for statistical analysis of the results. The one-factor *Analysis of Variance* (ANOVA) test followed by a *post-hoc* test (LSD) was performed to compare the means of the different groups. Pearson's correlation was used to study the relationship between the different variable parameters. All results with $p < 0.05$ were considered significant. The results were expressed as mean ± standard error on the mean.

3. RESULTS

3.1 DNA Concentration and Purity

Table 2 represented the quantity and purity of DNA extracted from blood and liver tissues.The amount of DNA extracted from the liver tissues was more concentrated and more pure compare to the DNA extracted from blood.

3.1.1 Global blood and hepatic DNA methylation profile

Fig. 1 illustrates the effect of AEAB on liver and blood DNA methylation profile. No significant difference was observed in the overall blood DNA methylation profile in all groups (NC, PC AEAB200, AEAB400, and Metformin). However, in the liver, global DNA methylation was significantly lower in the untreated diabetic group compared to the normoglycemic group (0.33% vs 0.61%). In the extract-treated groups, this methylation profile was significantly higher compared to the untreated diabetic rats' group (0.41% and 0.63% respectively versus 0.33%). Furthermore, the extract at 400mg/kg bw exhibited higher DNA methylation compared to the metformin-treated group ($p<0.05$).

Table 1. Dietary composition of the normal and high fat diet (Ble-Castillo et *al*., 2012) with some changes

Composition in g per 100 g of food		
Ingredient	**Normal diet (ND)**	**High fat diet (HFD)**
Fish meal	10	10
Milk	10	10
Refined palm oil	10	12.5
Margarine	/	12.5
Sucrose	/	18
Depulped cornflour	35	18
Wheat flour	20	15
Salt	1.5	1.5
Bone meal	2.5	2.5
Polyvitamins	1	1
Fibers(cellulose)	1	1
Distilled water	10	/

Table 2. Amount of DNA extracts and purity

Groups		Blood	Liver
NC	DNA concentration (ng/µL) Purity (%)	62.32±8.66(0.75)[a]	542.54 ±56.73 (1.74) [a]
PC	DNA concentration (ng/µL) Purity (%)	52.37 ±8.66 (0,85) [a]	441.19 ±56.73(1,62) [b]
AEAB 200	DNA concentration (ng/µL) Purity (%)	55.93 ±8.66 (0,80)[a]	398. 82 ±56. (1,68) [b]
AEAB 400	DNA concentration (ng/µL) Purity (%)	62.37 ±8.66(0,85)[a]	518.19 ±56.73 (1,70) [a]
REF	DNA concentration (ng/µL) Purity (%)	83.77 ±8.66 (0,78) [b]	506.22±56.73 (1,62) [a]

Values are expressed as mean ± standard deviation, NC: Negative control; PC: Positive control; AEAB200: group treated with 200mg/Kg BW of aqueous extract of A. boonei barks; AEAB400: diabetic rats + 400mg/Kg BW of aqueous extract of A. boonei barks; REF: diabetic rats + 10mg/Kg BW of metformin; values in parentheses represent the percentage of purity; values assigned different letters (a, b, c, d) are significantly different between groups at the same time (P<0.05).

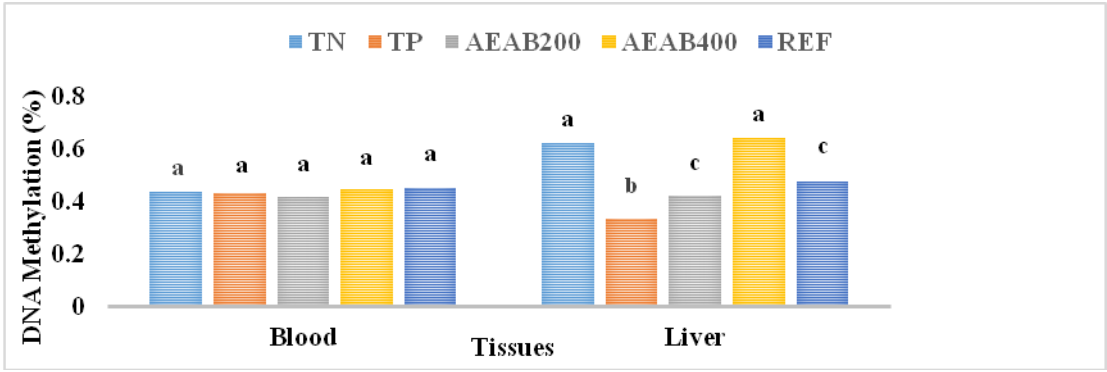

Fig. 1. Expression of global DNA methylation profile in liver and blood

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEAB200: group treated with 200mg/Kg BW of aqueous extract of A. boonei barks; AEAB400: diabetic rats + 400mg/Kg BW of aqueous extract of A. boonei barks; REF: diabetic rats + 10mg/Kg BW of metformin; Values assigned to different letters (a, b, c, d) are significantly different (P<0.05)

3.1.2 Effect of the extract on fasting hyperglycaemia

The effects of the extract on fasting hyperglycemia were expressed as mean blood glucose and percentage change as shown in Table 3. There is a significant increase in blood glucose with a mean of 554 ±26.69 mg/dL one day after administration of STZ to the rats as compared to normo glycemic group (negative control). After 28 days of treatment, the extract at both doses (200mg/kg CP and 400mg/kg CP) induced a significant decrease in blood glucose levels from day 14 (-20.89% and -22.74% respectively) today 28 (-24.87 and -29.87% respectively). The extract was more effective than metformin which only resulted in a reduction of -19.14% at day 28.

3.1.3 Association between global hepatic DNA methylation and blood glucose variation

A significant negative correlation (r = -0.90; p = 0.03) was found between the global hepatic DNA methylation and the blood glucose level among diabetic rats treated with a dose of 400 mg/kg bw of the aqueous extract of *A. boonei* (Table 4).

3.2 Hepatic Oxidative Status

3.2.1 Effect of the extract on the liver superoxide dismutase (SOD) activity

The hyperglycemic condition was associated with a significant decrease in liver SOD activity in untreated hyperglycemic rats as compared to normo glycemic group. The administration of the extract at 400mg/kg BW induced a significant increase of this activity compared to other groups as shown in Fig. 2.

3.2.2 Effect of the extract on the liver catalase activity

Fig. 3 reveals a significant ($P < 0.05$) decrease in liver catalase activity of PC group compared to NC group. Meanwhile, the administration of the extract at both doses was associated to an increase of catalase activities even compared to reference group which received metformin ($P < 0.05$).

3.2.3 Effect of the extract on the liver Malondialdehyde (MDA) level

Fig. 4 shows that the MDA level was significantly higher ($P < 0.05$) in the liver of the untreated diabetic group (PC) compared to the normo glycemic group; however, the administration of the extract resulted in a significant reduction of this maker of lipid peroxidation.

3.2.4 Association between global DNA methylation and oxidative stress in the liver

The association between global DNA methylation and some markers of oxidative stress was explored. It appears that no significant relationship was found between these oxidative stress markers and liver DNA methylation level (Table 5).

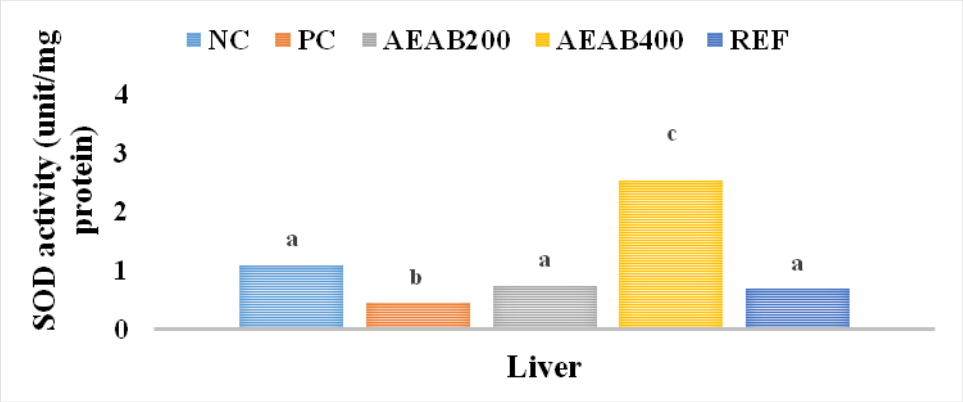

Fig. 2. Expression of hepatic SOD activity

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEAB200: group treated with 200mg/Kg BW of aqueous extract of A. boonei barks; AEAB400: diabetic rats + 400mg/Kg BW of aqueous extract of A. boonei barks; REF: diabetic rats + 10mg/Kg BW of metformin; Values assigned to different letters (a, b, c, d) are significantly different (P<0.05)

Table 3. Change in fasting blood glucose after STZ administration in rats

Groups		T=0 (24H after induction)	T=14 days	T=28 days
NC	Blood glucose (mg/dL)% variation	85.80±4.31(0)	87.40±3.52(1.86) [a]	87.40±3.52(1.86) [a]
PC	Blood glucose (mg/dL)% variation	548±30.79(0)	487.62±19.12(-11.11) [b]	487.1±19.12(-11.11) [b]
AEAB 200	Blood glucose (mg/dL)% variation	553.40±26.69(0)	429.33±2.66(-20.89) [c]	415.75±3.4 (-24.87) [c]
AEAB 400	Blood glucose (mg/dL)% variation	557.60±16.26(0)	430.75±15.18(-22.74) [c]	390±19.12(-29.87) [d]
REF	Blood glucose (mg/dL)% variation	558.40±20.74(0)	485±4.00(-13.14) [b]	451.5±18.5 (-19.14) [c]

Values are expressed as mean ± standard deviation, NC: Negative control; PC: Positive control; AEAB200: group treated with 200mg/Kg BW of aqueous extract of A. boonei barks; AEAB400: diabetic rats + 400mg/Kg BW of aqueous extract of A. boonei barks; REF: diabetic rats + 10mg/Kg BW of metformin values in parentheses represent percentage changes in blood glucose levels; values assigned different letters (a, b, c, d) are significantly different between groups at the same time (P<0.05).

Table 4. Correlation between global hepatic DNA methylation and blood glucose at day 28

	Blood glucose days 28	
	Correlation coefficient, r	Significance, p
DNA methylation (*AEAB200*)	-0.35	0.55
DNA methylation (*AEAB200*)	-0.90	0.03

AEAB: Aqueous extract of A. boonei barks; r: Correlation coefficient; Values of p<0.05 are considered significant

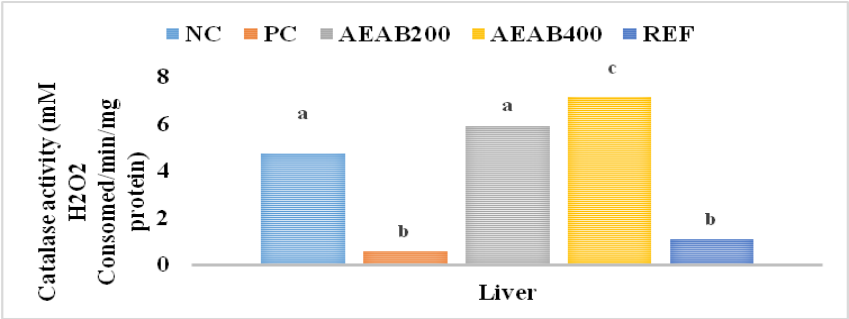

Fig. 3. Expression of hepatic catalase activity

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEAB 200: rats treated with 200 mg/Kg BW of A. boonei barks; AEAB 400: rats treated with 400 mg/kg BW of A. boonei barks; Reference (diabetic rats + 10mg/Kg BW of metformin). The values assigned with different letters (a, b, c, d) are significantly different (P< 0.05)

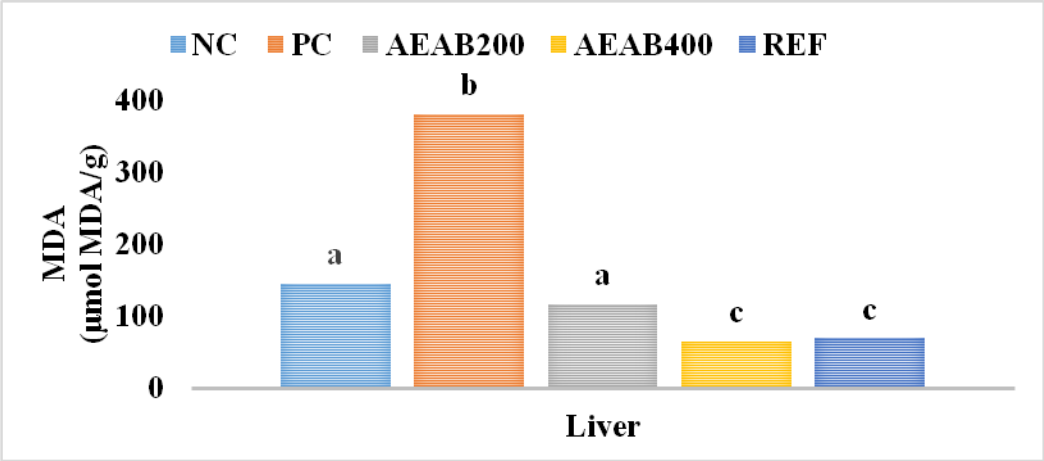

Fig. 4. Expression of hepatic lipid peroxidation

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEAB 200: rats treated with 200 mg/Kg BW of A. boonei barks; AEAB 400: rats treated with 400 mg/kg BW of A. boonei barks; Reference (diabetic rats + 10mg/Kg BW of metformin). The values assigned with different letters (a, b, c, d) are significantly different (P< 0.05)

Table 5. Correlation between global DNA methylation and liver endogenous oxidative stress markers

	SOD_{liver}		Cat_{liver}		MDA_{liver}	
	r	**p-value**	**r**	**p-value**	**R**	**p-value**
Methylation (*AEAB200*)	0.29	0.63	-0.13	0.83	-0.55	0.34
Methylation (*AEAB400*)	0.35	0.55	-0.02	0.97	-0.53	0.97

AEAB: Aqueous extract of A. boonei barks; r: Coefficient of correlation.

3.2.5 Plasma lipid profile

Hyperglycemic state resulted in an alteration of lipid profile in untreated rats' group (positive control) compared to the normo glycemic group (negative control) ($P < 0.05$) (Table 6). Rats receiving AEAB, exhibited a positive improvement of lipid markers (low triglycerides, total cholesterol VLDL-cholesterol and LDL-cholesterol level as well as an increase of HDL-cholesterol) as likely as metformin (a reference molecule).

3.2.6 Association between hepatic DNA methylation and plasma lipid profile

In Table 7, a strong positive correlation ($r=0.89$; $p<0.05$) was noted between the percentage of hepatic DNA methylation induced by the administration of the extract at the dose of 400 mg/kg BW and plasmatic HDL cholesterol level. For the other parameters, no significant correlation was observed.

3.2.7 Effect of the extract on atherogenic risks

Table 8 shows the effect of the extract on some artherogenic risks indexes (CRR, AC, AIP and CPI). The hyperglycemic state was associated with higher values of CRR, AC, API, and lower CPI in positive control group compared to the normal group *($p < 0.05$)*. Administration of the extract at both doses significantly reduced CRR, AC and AIP while increasing CPI in AEAB treated groups as compared to untreated group or reference group.

4. DISCUSSION

Several environmental factors such as diet and stress can affect the DNA methylation status and induce the development of diabetes and many of its complications [30]. In the diabetic liver in particular, there is an alteration in the overall DNA methylation profile materialized by hypomethylation [31]. Thus, one way to manage diabetes status is to prevent or correct the hypomethylation of hepatic DNA, an epigenetic reversible modification. We therefore conducted this study to evaluate the ability of the aqueous extract of *A. boneei* backs to reduce chronic hyperglycemia and prevent associated complication through the modulation of global hepatic DNA methylation.

Table 6. Expression of plasma lipid profile

	NC	PC	AEAB 200	AEAB 400	Reference
TG(mg/dL)	123.39±6.59[a]	322.18±9.80[b]	188.50±9.53[c]	129.50±3.18[a]	309.90±2.31[b]
TC(mg/dL)	126.72±5.19[a]	176.72±6.12[b]	133.49±5.50[a]	141.19±1.75[a]	177.04±8.64[b]
VLDL-C(mg/dL)	24.64±0.44[a]	64.40±0.80[b]	37.68±1.44[c]	25.90±2.22[a]	61.98±4.54[d]
LDL-C(mg/dL)	59.18±1.18[a]	80.17±0 .38[b]	42.26±0.64[c]	61.79±0.51[a]	59.12±1.82[a]
HDL-C(mg/dL)	42.23±1.18[a]	32.11±0.38[b]	53.51±0.64[c]	53.49±0.51[c]	55.93±1.82[c]

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEAB 200: rats treated with 200 mg/Kg of A. boonei barks; AEAB 400: rats treated with 400 mg/kg of A. boonei barks; Reference: diabetic rats + 10mg/Kg of metformin. TG: Triglycerides, TC: Total cholesterol, VLDL-C: Very low density lipoprotein cholesterol, LDL-C : Low density lipoprotein cholesterol, HDL-C : High density lipoprotein cholesterol. Values assigned different letters (a, b, c, d) on the same line are significantly different (P<0.05).

Table 7. Correlation between extract-induced global DNA methylation and lipid profile

	TG		c-VLDL		c-LDL		c-HDL	
	r	p-value	r	p-value	R	p-value	r	p-value
Methylation (*AEAB200*)	0,35	0,55	0,32	0,59	-0,64	0,24	0,06	0,91
Methylation (*AEAB400*)	-0,64	0,24	-0,69	0,20	0,61	0,34	**0,89**	**0,03**

AEAB: Aqueous extract of A. boonei barks; r: Coefficient of correlation; p-values represent significances (p < 0.05).

Table 8. Effect of the AEAB on atherogenic risk

	NC	PC	AEAB 200	AEAB 400	Reference
CRR1	3,00±0,16[a]	5,50±1,04[b]	2,49±0,85[a]	2,63±0,94[a]	3.16±1.01[ab]
CRR2	1,41±0,05[a]	2,49±0,85[b]	0,78±0,06[c]	1,15±1,1[a]	1,05±0,55[a]
AC	1,92±0,45[a]	9,03±2,66[b]	2,52±0,77[c]	1,42±0,52[a]	4,54±1,24[a]
AIP	0,46±0,001[a]	1±0,05[b]	0,40±0,001[a]	0,15±0,02[a]	0,65±0,001[b]
CPI	0,70±0,02[a]	0,40±0,01[b]	1,26±0,03[c]	0,86±0,06[a]	0,94±0,07[c]

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEAB 200: rats treated with 200 mg/Kg of A. boonei barks; AEAB 400: rats treated with 400 mg/kg of A. boonei barks; Reference: diabetic rats + 10mg/Kg of metformin. CRR: Cardiac Risk Ratio, AC: Atherogenic Coefficient, AIP: Atherogenic Index of Plasma, CPI: Cardioprotective Index. Values with different letters (a, b, c, d) on the same line are significantly different (P<0.05).

The results revealed that after 28 days of experimentation, hyperglycemia induced by intraperitoneal injection of STZ resulted in a significant *(p < 0.05)* reduction of the global methylation profile of the hepatic DNA of rats of the untreated diabetic group compared to the non-diabetic group (Figure 1). This is consistent with Williams et al*.* [32] results whose found that after 8 weeks, rats with chronic hyperglycemia induced by STZ, presented hepatic DNA hypomethylation. Induced hyperglycemia would have generated oxidative stress which stimulated poly(ADP-ribose) polymerase activity in the liver of these rats, thus activating the active DNA demethylation pathway involving ten-eleven-translocation(Michele et *al.*, 2021). The significant increase of the hepatic DNA methylation profile among rats receiving AEAB (especially 0.63% at the 400mg/kg BW dose, as well as the negative strongly correlation with blood glucose (r = -0.90; P= 0.03) (Table 4) would be due to the AEAB could have reduced chronic hyperglycemia by increasing the overall methylation profile of hepatic DNA; which would have reduce the expression of the *PKCε* gene involved in hepatic insulin resistance [7]. This beneficial effect of the extract could be due to justified by the presence of polyphenols especially flavonoids which have the abilities to increase DNA methyltransferase activity at the hepatic level and inhibit poly(ADP-ribose) polymerase [33] (Laszlo et *al*., 2021). Moreover,

although the rate of DNA methylation induced by the extract at the dose 200 mg/kg BW (0.41%) was not significantly correlated with the decrease in blood glucose (Table 4); the presence of alkaloids in the extract could have significantly decrease in blood glucose in treated groups compared to the PC group at days 14 and days 28 (Table 3) this through the inhibition of gluconeogenesis enzymes [34]. Indeed, Akinloye et al. [35] had revealed that the aqueous extract of *A. boonei* barks reduced the activity of hepatic glucose-6-phosphatase and glucose-1,6-biphosphatase.

The alteration of the global DNA methylation profile induced by chronic hyperglycemia has been implicated in the phenomenon of metabolic memory [30]. Indeed, these aberrant profiles persist after blood glucose levels are restored to normal and contribute to micro and macrovascular complications of diabetes [36]. Thus, the effect of the extract on the overall hepatic DNA methylation profile would be predictive of its protective role on hyperglycemia-related complications [37,38,39]. It has been shown that in diabetic patients, hepatic DNA hypomethylation was associated with chromatin instability and liver cancer [40-43]. Results obtained show that the extract could be a good candidate in the prevention of hepatic carcinoma in diabetic subjects. Since DNA methylation is involved in the inhibition of gene expression (Long et *al*., 2018), hepatic SOD and Catalase activities were improved in rats' groups receiving the extract compared to the untreated diabetic one (figure 2; figure 3), This result could reflect the fact that the global DNA methylation induced by the extract had no repressive effect on the expression levels of the hepatic SOD and catalase gene, thus showing the specificity of action of the extract. This was further confirmed by the absence of significant correlation between the methylation induced by the extract and the activities of these two antioxidant enzymes (Table 5). Furthermore, the increased activities of these two enzymes in AEAB-treated groups could therefore be attributed to the fact that the global DNA methylation induced by the extract could have affected the loci of the *KEAP1* gene, thus reducing its expression [44]. Indeed, *KEAP1* protein is known to bind to Nrf2-related factor 2 and promote its degradation. Nrf2 is known to activate several antioxidant enzymes such as SOD and Catalase [45]. On the other hand, Afolabi et al. [46] identified in *A. boonei* barks the presence of Iron which is a cofactor (activator) of Catalase [47]. The ability of this extract to improve the antioxidant status, would therefore explain the protection of lipid membranes against lipid peroxidation hence the low levels of MDA noted among AEAB treated rats' (Fig. 4).

As dyslipidemia is one of the metabolic disorders associated with diabetic type 2 state, evaluation of the effect of the AEAB on lipid profile markers in relation to global hepatic DNA methylation as well as on atherogenic risk revealed an alteration of lipid metabolism among untreated diabetic rats (Table 6). This could be a consequence of the altered global hepatic DNA methylation profile. Indeed, global hypomethylation of hepatic DNA has been associated with altered expression of genes involved in lipid metabolism [48]. Administration of the AEAB resulted in a decrease in triglycerides, total cholesterol, LDL-C, VLDL-C and an increase in HDL-C (Table 6). This suggests that the AEAB reduces diabetic dyslipidemia by correcting global hypomethylation of hepatic DNA. This was further confirmed by the strong and positive correlation ($r = 0.89$; $P = 0.03$) between global DNA methylation level and HDL-C at the 400mg/kg BW of the extract (Table 7). Mice with a hypomethylation genome had eleveted expression of inflammatory markers and DNA hypomethylation was shown to precede the formation of aortic fatty streaks [49,50].Such changes were shown to contribute to promote atherosclerosis and hypertension [48]. Thus, these beneficial effects of the extract at the level of the methylation of hepatic DNA, are reflected by the decrease in cardiac risk ratio, (CRR) coefficient(AC)atherogenic index of plasma(AIP) and increase incardioprotective index(CPI) in AEAB-treated group (Table 8). The global DNA methylation induced by the extract could have affected the expression levels of genes involved in HDL-C catabolism such as the cholesterol ester transfer protein (CETP) gene. Indeed, Guay et al. [51] showed that methylation of this gene was associated with high levels of plasma HDL cholesterol. The hypotriglyceridemic and hypocholesterolemic properties of the extract could be attributed to their polyphenol content, which are capable of stimulating lipoprotein lipase and inhibiting HMG-CoA reductase, key enzymes involved in triglyceride catabolism and cholesterol synthesis respectively [52].

5. CONCLUSION

The aqueous extract of *Alstonia boonei* bark reduces chronic hyperglycemia and prevents associated complications by increasing

methylation of hepatic DNA. This work further supports the multiple beneficial effects of *A. boonei* in the management of diabetes-related metabolic disorders. However, further works are needed to evaluate the effect of the extract on the catalytic activity of poly(ADP-ribose) polymerase as well as on intermediates of the active DNA demethylation pathway such as 5-hydroxylmethylcytosine and 5-carboxylmethylcytocin in the liver.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wells JC. The evolution of human fatness and susceptibility to obesity: An ethological approach. Biol Rev Camb Philos Soc. 2006;81:183-205.
2. American Diabetes Association. Standards of medical in diabetes. Diabetes care. 2020;43:14-3.
3. International Diabetes Federation (IDF). People living with diabetes. 9th Edition. Diabetes Atlas.2019;4.
4. International Diabetes Federation (IDF member(s) In Cameroon). Prevalence of Diabetes in adults;2020.
5. Fahmida A, Asiful I, Siew H, Mafauzy M, Teguh H. DNA Methylation: An Epigenetic Insight into Type 2 Diabetes Mellitus. Current Pharmaceutical Design. 2016;22:1-21.
6. Monhoval P, Lotstra F. Transmission intergénérationnelle des caractères acquis épigénétiquement. Cahiers de psychologie Clinique. 2014;43:39-42.
7. Du Q, Luu PL, Stirzaker C, Clark SJ. Methyl-CpG-binding domain proteins: readers of the epigenome. Epigenomics. 2015;6:73-1051.
8. Sarras M, Robert V. The role of DNA methylation in type 1 and 2 diabetes as related to endothelial cell dysfunction. Journal of Anatomy and Physiology. 2015;1:20-25.
9. Lee J, Friso S, Choi S. Epigenetic mechanisms underlying the link between non-alcoholic fatty liver diseases and nutrition. Nutrients.2014;6:3303-3325.
10. Kirchner H, Sinha I, Gao H, Ruby M, Schönke M, Lindvall J, Barrès R, Krook A, Näslund E, Dahlman-Wright K, Zierath J. Altered DNA methylation of glycolytic and lipogenic genes in liver from obese and type 2 diabetic patients. Molecular Metabolism. 2016;5:171-183.
11. Hota B, Ellenbogen C, Hayden MK, Aroutcheva A, Rice TW, Weinstein RA. Community-associated methicillin-resistant Staphylococcus aureus skin and soft tissue infections at a public hospital: do public housing and incarceration amplify transmission. Arch Intern Med. 2007;167:33-1026.
12. Rauter A, Martins A, Lopes R, Ferreira J, Serralheiro L, Araujo M, Mota-Filipe H. Bioactivity studies and chemical profile of the antidiabetic plant Genista tenera. Journal of Ethno pharmacology. 2009;2:93-384.
13. Morand C, Milenkovic D. Polyphenols and vascular health: evidence for a direct role of polyphenols in the beneficial effects of citrus in vascular protection. Agronomic Innovations. 2014;42:47-62.

14. Filipa M, Vania C, Victor G, Joao F, Rui H, Carmen J. Repurposing Old Drugs into New Epigenetic Inhibitors: Promising Candidates for Cancer. Treatment? Journal of pharmeuceutics. 2020;12:410.
15. Babamale OA, Lyiola OA, Adeyemi SB, Sulyman FA, Abdulkareem AO, Anifowoshe AT, Awe OD, Ajani D, Ugbomoiko U. Comparative studies of genotoxicity and anti-plasmodial activities of stem and leaf extracts of Alstonia boonei (De Wild) in malaria-infected mice. Nigerian Journal of Parasitology.2017;38(2):1117-4145.
16. Funmilola O, Taiwo F. Antiplasmodial activity of stem bark and leaves of Alstonia boonei (De Wild). Journal of Microbiology and Experimentation. 2019;7:5.
17. Gbadamosi I, Moody J, Lawal A. Phytochemical screening and proximate analysis of eight ethnobotanicals used as antimalaria remedies in Ibadan. Nigeria. Journal. Apply. Bioscience. 2011;44:2967-2971.
18. Owolabi OJ, Arhewoh IM, Innih SO, Anaka ON, Monyei CF. The ethanol leaf extract of alstonia boonei (apocynaceae) reduces hyperglycemia in alloxane-induced diabetic rats. Nigerian Journal of Pharmaceutical Sciences. 2014;13:12-21.
19. Barnabé L, Selestin D, Dzeufiet D, Pierre K. Antihyperglycemic and antioxidant properties of *Alstonia boonei* De Wild. (Apocynaceae) stem bark aqueous extract in dexamethasone-induced hyperglycemic rats. International Journal of Diabetes Research. 2014;3:27-35
20. Ajose DJ, Onifade OF, Wemambu I. Phytochemical analysis and in vitro antibacterial evaluation of leaf and bark extracts of Alstonia boonei. African Journal of Pharmacy and Pharmacology. 2019;13:287-291.
21. Ble-Castillo J, Aparicio-Trapala M, Juárez-Rojop E, Torres-Lopez J, Mendez J, Aguilar-Mariscal H, et Diaz-Zagoya J. Differential effects of high-carbohydrate and high-fat diet composition on metabolic control and insulin resistance in normal rats. International Journal of Environmental Research and Public Health. 2012 ;9:1663-1676.
22. Umashanker N, Vaibhav G, Sheth S, Kulbhushan T. Dietary Supplementation of Methyl Donor L-Methionine Alters Epigenetic Modification in Type 2 Diabetes. Molecular nutrition. 2019;63:1-11.
23. Chang KJ. Effect of taurine and beta alanine on morphological changes of pancreas in streptozotocin induced diabetic rats. Experimental Biology and Medicine. 2000;483-571.
24. Plowe CV, Djimde A, Bouare M, Doumbo O, Wellems TE. Pyrimethamine and Proguanil Resistance-Conferring Mutations in Plasmodium falciparum Dihydrofolate Reductase: Polymerase Chain Reaction Methods for Surveillance in Africa. The American Journal of Tropical Medicine and Hygiene. 1995;52:565-568.
25. Coombs NJ, Gough AG, Primrose JN. Optimization of DNA and RNA extraction from archival formalin-fixed tissue. Nucleic acids Research. 1999;27(16).
26. Yagi K. Simple Fluorometric Assay for lipoperoxide in blood plasma. Biochemistry Medicine. 1976;15:212-216.
27. Misra H, Fridowich I. The generation of superoxide radical during the autoxidation of hemoglobin. Journal of Biological Chemistry. 1972;247:6960-6962.
28. Sinha K. Colorimetry assay of catalase. Analytical Biochemistry. 1972;47:389-394.
29. Friedewald W, Levy R, Friedrickson D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18(6):499-502.
30. Ikewuchi J, Ikewuchi C. Alteration of plasma lipid profiles and atherogenic indices by Stachytarpheta jamaicensis L. (Vahl). Biochemistry. 2009a;21(2):71-7.
31. Ikewuchi J, Ikewuchi C. Alteration of plasma lipid profile and atherogenic indices of cholesterol loaded rats by Tridax procumbens Linn: Implications for the management of obesity and cardiovascular diseases. Biochemistry. 2009b ;21(2): s95-9.
32. Althunibat O, Al Hroob A, Abukhalil M, Germoush M, Bin-Jumah M, Mahmoud A. Fisetin ameliorates oxidative stress, inflammation, and apoptosis in diabetic cardiomyopathy. Life Science. 2019;15(221):83-92.
33. šoli'c N, Goluža E, Dikic' D, Lisiˇci' D, Sašilo K, Rođak E, Jeleˇc Ž, Lazarus M, Orct T. Role of flavonoids on oxidative stress and mineral contents in the retinoic

acid-induced bone loss model of rat. European Journal of Nutrition. 2014;53:1217-1227.

34. Kowluru R. Diabetic retinopathy, metabolic memory and epigenetic modifications. Visual Research. 2017;139:30-38.
35. Lai Z, Chen J, Ding C, Wong K, Chen X, Pu L, Huang Q, Chen X, Cheng Z, Liu Y. Association of Hepatic Global DNA Methylation and Serum One-Carbon Metabolites with Histological Severity in Patients with NAFLD. Journal of Obesity. 2020;28:197-205.
36. Williams K, Timothy A, Garrow, Kevin L. Type I Diabetes Leads to Tissue-Specific DNA Hypomethylation in Male Rats. The Journal of Nutrition. 2008;138:2064-2069.
37. Ighodaro OM, Akinloye OA. First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defense grid. Alexandria Journal of Medicine. 2017;54:287-293.
38. Barajas-Olmos F, Centeno-Cruz F, Carlos Z, Imaz-Rosshandler I, Martínez-Hernández A, Emilio J, Rangel-Escareño C, Faustino G, Armando C, Hernán M, Francisco C, Maldonado-Pintado D, Lorena O. Altered DNA methylation in liver and adipose tissues derived from individuals with obesity and type 2 diabetes. BMC Medical Genetics. 2018;19-28.
39. Michele Z, Maria GB, Ilaria B, Stefania S, Flavia AC, Laura B, Stefano T, Giovanna D, Giuseppe Z, Maria GC, Anna R. Increased PARylation impacts the DNA methylation process in type 2 diabetes mellitus. Clinical Epigenetic. 2021;13:114.
40. Chen Z., Karaplis A.C. and Ackerman S.L. (2001). Mice deficient in methylenetetrahydrofolate reductase exhibit hyper homocysteinemia and decreased, with neuropathology and aortic lipid deposition. Human molecular genetics.10.433-443.
41. Kenneth W, Paola S, Paul J, Simin L, Dawn D, José O. DNA methylation modules associate with incident cardiovascular disease and cumulative risk factor exposure. Clinical Epigenetics. 2019;11-142.
42. Gay SP, Brisson D, Lamarche D, Vohl MC, Gaudet D, Bouchard D. Variation of DNA methylation in the loci of CETP and LPL genes: news molecular biomaquars associated with plasma lipids profile. Journal of atherosclerosis. 2013;228:413-420.
43. Abdulazeez E, Wudil A, Yunusa A. Effect of partially purified angiotensin converting enzyme inhibitory proteins from moringa oliefera leaves on alloxane induced diabetic rats. Ife Journal of Science. 2011;19(1).
44. Junko M, Erica J, Colleen A, Mitsuru U, Yasushi A, Takamasa A. Natural and glucose flavonoids inhibit poly(ADP-ribose) polymerase activity and induce synthetic lethality in BRCA mutant cells. Oncology Reports. 2014;31:551-556.
45. Laszlo S, Richard M, Andras T, Arpad D, Richard D, Ghodratollah N, Timea V, Balazs N, Ferenc B. et Istvan K. The effects of flavonoids, green tea polyphenols and coffee on DMBA induced LINE-1 DNA hypométhylation. PLOS ONE. 2021;16(4):157-250.
46. Xuan X, Jinhua Y, Yunfeng S, Kuanxiao T, Jun Y, Yanhua Z, Dongjie Y, Hua L, Jianping Y, Jianping W. Berberine Improves Glucose Metabolism in Diabetic Rats by Inhibition of Hepatic Gluconeogenesis. Plose One. 2011;6:16556.
47. Akinloye OA, Oshilaja RT, Okelanfa OA, Akinloye DI, Idowu OM. Hypoglyceamic activity of Alstonia boonei stem bark extract in mice. Agriculture and Biology Journal of North America. 2013;4:1-5.
48. Long H, Yin H, Wang L. The epigenetics of autoimmunity: An overview. Academic Press. 2018;5:1-23.
49. Jeongeun H, Youngmi J. DNA Methylation in Nonalcoholic Fatty Liver Disease. International journal of molecular science. 2020;21:8138.
50. Dong F, Wang S, Wang Y, Yang X, Jiang J, Wu D, Qu X, Fan H, Yao R. Quercetin ameliorates learning and memory via the Nrf2-ARE signaling pathway in d-galactose-induced neurotoxicity in mice. Biochemistry Biophysics Research. Common. 2017;491:636–641.
51. Mallique Q, Jian X, Yuejun Y, Yuancai L, Shugeng C. Natural Nrf2 Activators from Juices, Wines, Coffee, and Cocoa. Beverages. 2020;6:68.

52. Afolabi C, Ibukun EO, Emmanuel A, Akinrinlola TR, Onibon AO, Akinboboye EM, Obuotor and Farombi EO. Chemical constituents and antioxidant activity of Alstonia boonei. African Journal of Biotechnology. 2007;6:1197-1201.

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