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Effect of *Plumeria alba* Linn. (Apocynaceae) fractions on some parameters of high fat diet induced metabolic syndrome in mice

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Abstract
The root of *P. alba* is traditionally used to treat type 2 diabetes. The aim of this study was to evaluate the preventive effect of *P. alba* fractions on some markers of metabolic syndrome induced by lard- fructose diet in ICR mice. The mice were fed with lard - fructose mixture for 28 days, from the 15th to 28th day, 30 minutes before mice were given distilled water (Hyperlipidemic control), metformin (100 mg/kg), Ethyl Acetate fraction or Supernatant fraction (100 mg/kg per day) for the treatment. After 28 days of the experiment, fasting blood glucose, body weight gain, intra-abdominal fat, serum triglycerides (TG) and total cholesterol levels in the treated groups were significantly (p<0.01) lower than that of Hyperlipidemic control group. In the glucose tolerance test, treated group showed a significant (p<0.01) reduction of blood glucose during 180 minutes after glucose loading, which indicates that the fractions of *P. alba* improve glucose tolerance. The finding in this study reveals that *P. alba* fractions can prevent metabolic syndrome induced by lard-fructose diet in mice.

Keywords: *Plumeria alba;* Fraction; metabolic syndrome; Diabetes; Lipid profile.

Introduction
Obesity has become a worldwide epidemic in the past decades, results from excessive energy intake and a lack of physical exercise, [1] metabolic disorders including hyperinsulinemia, impaired glucose tolerance and dyslipidemia are often associated with obesity, which increases the risk for several chronic diseases such as cardiovascular disease, stroke, type 2 diabetes and hypertension [2-4]. The metabolic syndrome is a complex of interrelated risk factors for cardiovascular disease and diabetes. These factors include hyperglycemia, raised blood pressure, elevated triglyceride levels (TG), decreased high density lipoprotein cholesterol level (HDL-C), and obesity. It has been demonstrated clearly that this syndrome is common and that it has a rising prevalence worldwide, which relates largely to increasing obesity and sedentary lifestyles. As a result, the metabolic syndrome is now both a public health and a clinical problem [5-7]. This increasing factors risk may be also due to excessive fructose consumption [8].

Despite the great of research about new synthetic drugs, Herbal medicines are used to treat diseases because of their efficiency, less side effects and relative low cost. In this way, *Plumeria alba*, belonging to the family Apocynaceae, is a tall tree of Togo flora, measuring about 12 m and 1 m in height and girth. Decoction of *P. alba* root is used in folk medicine to treat hyperlipidemia, type 2 diabetes mellitus and to manage obesity and the latex to treat wound [9]. In our previous study, the effects of hydroethanolic extract were proven on some parameters of type 2 diabetes and active fractions were isolated [10,11], there was lack of apparent toxicity, acute or sub-chronic, at doses greater than those that induce an effect in animal disease models [12].

The purpose of this study was to evaluate the effect of *P. alba* active fractions on some parameters of metabolic syndrome in mice fed with Lard fructose mixture.

2. Materials and Methods
Plant material
P. alba roots were collected from the garden of the Teaching Hospital Sylvanus Olympio of Lomé, Togo. A specimen was identified by the Laboratory of Botany and Plant Ecology

(Faculty of Science/University of Lomé) and retained in the department herbarium under number 8035. The roots were washed, dried under air-conditioning and reduced to powder with electric mill (Thomas Scientific™, 3375-E20). The powder was cold extracted in ethanol 95°/water mixture (80:20) for 72 h. The crude extracts were filtered with Whatman paper (N° 1) and evaporated under vacuum at 45 °C using a rotary evaporator Büchi R210. The yield of the preparation was 11.34%.

Fractionation of hydro alcoholic extract

For fractions, we dissolved 30 g of total extract in 400 ml of 75% alcohol. The solution was dispensed into 10 ml tubes and left in a refrigerator at 4 °C for 24 hours to allow the precipitate to settle at the bottom of the tube. The supernatant was separated from the clot by centrifugation at 2500 g and then evaporated under vacuum at 45 °C to obtain supernatant fraction yield of 73.33% (SF). The ethyl acetate fraction (EA) was obtained by dissolving 30 g of total extract in 10 mL of distilled water and then in 400 ml of ethyl acetate. The mixture was refrigerated, treated as above and the ethyl acetate phase was evaporated to give ethyl acetate fraction with a yield of 82.2%.

Animals: Male mice weighing 20-30 g were provided from the Animal House of physiology/pharmacology department of the University of Lomé (Togo). Animals were maintained at the standard environmental conditions (temperature of 25 ± 2 °C and 12/12h of light/dark cycle). They were given standard commercial rat chow and water *ad libitum*.

Chemicals: Products used for the pharmacological tests were purchased from the laboratory Sigma (St. Louis, Mo, USA, Richmond, CA, USA). The assay of total cholesterol and triglyceride were made using assay kits Labkit Chemelex-SA (Barcelona, Spain).

Effect of the SF and EA fraction on the model of metabolic syndrome in mice

for this protocol, the method described by Bakoma *et al*., [13] was modified by mixing fructose with lard.

Preparation of Lard-fructose mixture (L-F)

To 100 ml of L-F mixture, 6 g of fructose and 1.25% tween 80% were dissolved in 50 ml of distilled water, at the mixture was added 50 ml of molten lard.

Experimental protocol

35 male mice divided into 5 groups of seven (07) mice each. Animals were treated for 28 days following the procedure below:

- **Group I:** NC (normal control) animals received distilled water 5 ml/kg/day for 28 days. At the 15th to 28th day, animals received 30 minutes in advance, distilled water at a rate of 5 ml/kg/day;
- **Group II:** HC (hyperlipidemic control) animal received L-F mixture 5 ml/kg/day for 28 days. From the 15th to 28th day, mice received 30 min before administration of L-F mixture, distilled water 5 ml/kg/day;
- **Group III:** (HC+EA) and **IV** (HC+SF) received L-F mixture 5 ml/kg body weight for 28 days. From the 15th to 28th day, animals received 30 min before administration of the L-F mixture, respectively ethyl acetate fraction (EA) and the supernatant (SF) at a dose of 100 mg/kg/day;
- **Group IV:** (reference group) received L-F mixture to 5 ml/kg/day for 28 days. From 15th to 28th day, animals received 30 min before administration of L-F mixture, metformin 100 mg/kg/day.

Animal's weight was measured every 2 days and blood glucose every 7 days using one touch Ultra [2] glucometer. At the end of the experiment (day 28), animals were fasted for 14 hours. At the 29th day, animals were subjected to Oral glucose tolerance test (OGTT) by oral administration of glucose 2 g per kg body weight. Blood glucose was measured at 0, 30, 60, 120 and 180 minutes after glucose administration. The blood was collected at the retro orbital sinus after ether anesthesia and centrifuged at 3000 g for 10 minutes. The serum was collected in tubes for the determination of triglycerides, cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using Labkits. Mice were sacrificed by cervical dislocation. The abdominal cavity was opened and intra-abdominal fat and liver were removed and weighed.

Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). Statistical Analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test to evaluate significant differences between groups. $p<0.05$ was considered statistically significant. All statistical analyses were carried out using the instat statistical package (Graph Pad Software Inc., USA).

3. Results

Effect of fractions (SF and EA) on the weight gain

Hyperlipidemic control (HC) group presented after 28 days of treatment, a weight gain of 11.95±0.22% against 3.84±0.64% in normal control (NC). The administration of the ethyl acetate fraction (EA), the supernatant fraction (SF) and metformin induced a significant reduction in weight gain compared to hyperlipidemic control group, respectively 6.90±0.67% ($p<0.05$); 5.65±0.59% ($p<0.001$) and 4.75±0.67% ($p<0.001$) compared to HC group 11.95±0.22% (Fig 1).

Effect of fractions (SF and EA) on intra-abdominal fat

Three old higher fat accumulation was noted in HC group (10.52±0.63%) compared to NC group (3.90±0,66%). The group treated with EA at a dose of 100 mg/kg/day showed a significant reduction ($p<0.05$) of relative intra -abdominal fat weight (6.75±0.75%) compared to HC group (10.52±0.63%). Animals treated with SG and metformin at a the dose of 100 mg/kg have also respectively a significant reduction of fat accumulation 5.60±0.66% ($p<0.01$) and 4.77±0.66% ($p<0.001$) compared to HC animals (Fig 2).

Effect of fractions (SF and EA) on the relative liver weight

The relative liver weight was 2.81±0.10 g in the NC group against 3.35±0.12 g in HC animals. Animals treated with ethyl acetate supernatant and metformin at the dose of 100 mg/kg,

showed a significant (P<0.05) reduction of relative liver weight respectively 2.65±0.10%; 2.83±0.09% and 2.58±0.10% compared to HC group 3.35±0.12 g (Fig 3).

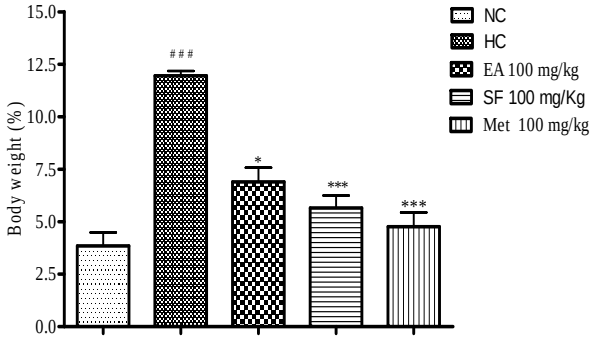

Fig 1: Effect of fractions of the weight gain

The weight gain was calculated from the formula (initial weight-final weight/initial weight) X 100. NC group received distilled to 5 ml/kg/day for 28 days water groups (HC, EA, SF and Met) were subjected to a hyperlipidemic treatment enriched with fructose for 28 days. Fractions and metformin were administered from the 15th to 28th day. Each value represents the mean ± SEM. ### P<0.001 (*vs* NC); * p<0.05; *** p<0.001 (*vs* HC).

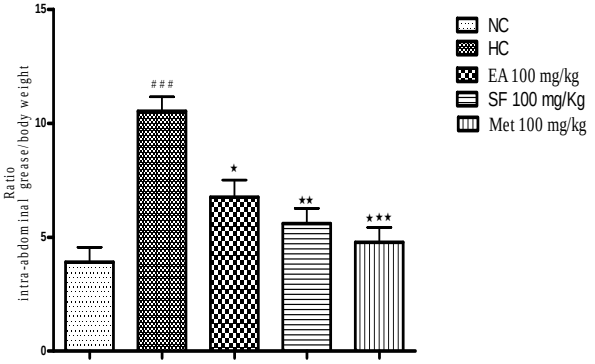

Fig 2: Effect of fractions of intra-abdominal fat

NC group received distilled water 5 ml/kg/day for 28 days; groups (HC, EA, SF and Met) were subjected to a hyperlipidemic treatment enriched with fructose for 28 days. Fractions and metformin were administered from the 15th to 28th day of the protocol. Intra-abdominal fat was dissected and weighed on the 29th day. Each value represents the mean±SEM. ### P<0.001 (*vs* NC); * P<0.05; ** P<0.01; *** p<0.001 (*vs* HC).

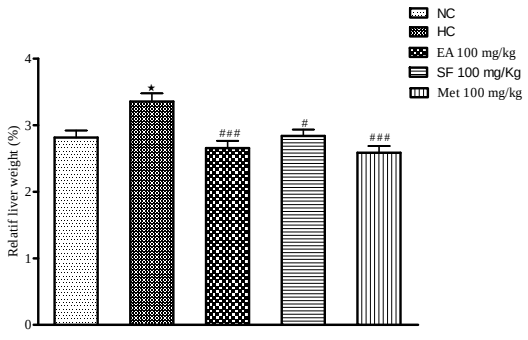

Fig 3: Effect of fractions on the relative liver weight

NC group received distilled water 5 ml/kg/day for 28 days, groups (HC, EA, SF and Met) were subjected to a hyperlipidemic treatment enriched with fructose for 28 days. Fractions and metformin were administered from the 15th to 28th day of the protocol. The relative liver weight is the weight ratio of liver taken from the final weight of the animal once cent. The results represent the mean±SEM. * p<0.05: #p<0.05 vs (NC); (HC); ### P<0.001: (*vs* HC).

Effect of fractions (SF and EA) on basal glucose level during the experiment

At Day 0, average basal blood glucose of animals was 80 mg/dl. After 14 days of hyperlipidemic diet, the HC group showed a basal blood glucose level of 234.57±10.65 mg/dl against 89.28±2.99 mg/dl in the NC group. The administration of ethyl acetate fraction, supernatant and metformin 100 mg/kg/day from the 15th to 28th day resulted in a significant reduction of the basal glucose level to 174.71±2.06mg/dl, 158.00±6.24mg/dl, 138.14±6.75mg/dl, respectively at 21th day to 172.71±2.12 mg/dl, 147.57±4.82 mg/dl and 126.42±4.84 mg/dl, respectively at 28th days (Fig 4).

Effect of fractions (SF and EA) on Oral Glucose Tolerance Test (OGTT)

After 28 days of treatment, the animals were subjected to OGTT with 2 g/kg of glucose and blood glucose was measured during 120 minutes. Thirty (30) minutes after the administration of glucose, the hyperlipidemic control (HC) group had a blood glucose level of 410.20±4.91 mg/dl±9.66 against 303.20 mg/dl in controls normal. The animals pretreated with the supernatant fractions and ethyl acetate showed a significant (p<0.001) reduction of blood glucose level compared to HC (A). The area under the curve (AUC) has shown that the supernatant and ethyl acetate reduced significantly (p<0.001) the amount of glucose circulating (A)° (Fig 5).

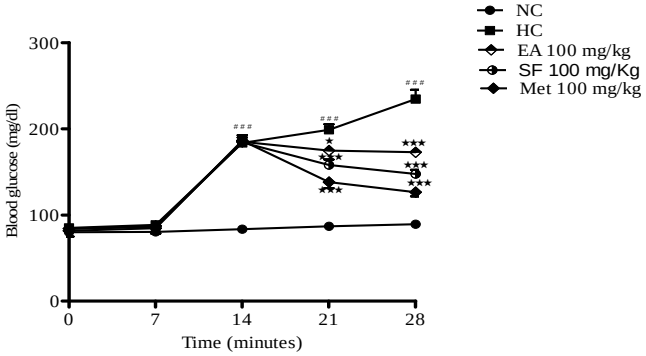

Fig 4: Effect of fractions on the evolution of the basal glucose level

NC group received distilled water for 28 days, HC groups, EA, SF and Met received an L-F diet for 28 days. These groups received respectively from the 15th to 28th day of the ethyl acetate fraction (100 mg/kg/day), the supernatant fraction (100 mg/kg/day) and metformin (100 mg/kg/day). Basal blood glucose was measured every 7 days after 14 hours of fasting. Each value represents the mean ± SEM. ### P<0.001 (*vs* NC), * p<0.01; *** p<0.001 (*vs* HC).

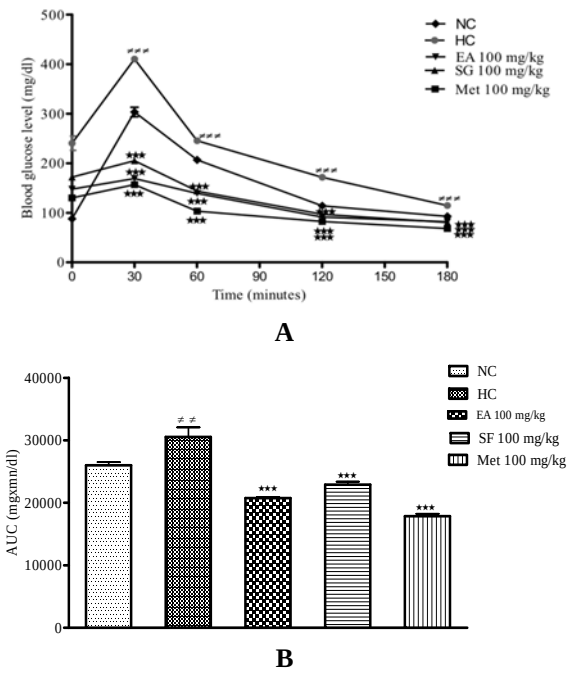

Fig 5: Effect of fractions on Intolerance glucose test

A: blood glucose variation during the experiment, **B:** Area under the curve of blood glucose level NC group received distilled water for 28 days, groups (HC, EA, SF and Met) received L-F mixture for 28 days. These groups received respectively from the 15th to 28th day of the ethyl acetate fraction (100 mg/kg/day), the supernatant (100 mg/kg/day) and metformin (100 mg/kg/day). At day 29, the animals were subjected to HPVO with 2 g/kg; glucose was measured for 3 hours. Each value represents the mean ± SEM. ## P<0.01; ### P<0.001 (*vs* NC); *** P<0.001 (*vs* HC).

Effect of fractions (SF and EA) on biochemical parameters

The administration of a L-F mixture led to a significant increase in AST (p<0.001), TG (p<0.01) and total cholesterol (p<0.01) but has no significant effect on ALT compared to normal control group. The administration of ethyl acetate fraction at 100 mg/kg/days for 14 days showed a significant reduction in the concentration of AST, TG and total cholesterol (p<0.01) compared to HC group. The supernatant fraction significantly reduced TG and total cholesterol (p<0.05) but had no effect on AST (table 1).

Table 1: Effect of fractions on the biochemical parameters measured

Parameters	NC	HC	EA 100 mg/kg	SF 100 mg/kg	Met 100 mg/kg
AST (UI/l)	106±0.87	271.2±4,38###	154,2±2.01**	158±21*	164±21*
ALT (UI/l)	64±7.2	71±6.1	48±10**	55±10*	63±7,7*
TG (mg/dl)	101±5.6	168±13##	88±9**	98±24*	93±5**
Ch (mg/dl	109±2.8	135±5. 9##	92±8.7**	112±10*	105±10**

NC group received distilled water for 28 days; groups (HC, EA, SF and Met) received a fructose-enriched to 60% for 28 days' supply. These groups received respectively from the 15th to 28th day of our experiments ethyl acetate fraction (100 mg/kg/day), the supernatant fraction (100 mg/kg/day) and metformin (100 mg/kg/day). The 29th day, blood was drawn for determination of biochemical parameters using labkit. Each value represents the mean ± SEM. ## P<0.01; ### P<0.001 (vs TN); * p<0.05; ** P<0.01 (*vs* HC)

Discussion

Fructose is a monosaccharide which can induce metabolic disorders such as glucose intolerance, hypertension, and dyslipidemia and plays a key role in the pathophysiological development of type 2 diabetes and atherosclerosis [14]. Our results are in agreement with previous studies [15, 16] who found that the consumption of diets rich in fructose induced an increase of blood glucose level associated with dyslipidemia and therefore, a reduction in insulin sensitivity. We observed glucose intolerance in mice by carbohydrate loading; reflecting the decreased ability of insulin to stimulate glucose disposal in peripheral tissues.

The hyperlipidemic diet (lard - fructose) induced in untreated animals an increase of body weight, due to the accumulation of intra-abdominal fat and an increase of relative liver weight compared to normal rats. These results suggest that mice fed with a diet develop a hyperlipidemic syndrome similar to that seen in human's abdominal obesity [17]. Our results showed that the administration of ethyl acetate fractions and supernatant of *P. alba* at doses of 100 mg/kg significantly reduced the weight gain, intra-abdominal fat accumulation and relative liver weight in hyperlipidemic treated mice compared to hyperlipidemic controls, **t**hose fractions were also the most active in our previous study [11]. Similar results were obtained in hyperlipidemic rats with *Bridelia ferruginea* hydroalcoholic extract [13]. This suggests that plant could contain active ingredients which are capable to act on lipid metabolism and mechanism of action and pass either an inhibitory effect on lipid absorption or stimulating lipolysis. The ethyl acetate fractions and supernatant as metformin significantly reduced hyperglycemia induced by the administration of fructose -lard in mice; oral glucose tolerance test showed a state of glucose intolerance in hyperlipidemic rats. Glucose intolerance was corrected by the administration of EA and SF fractions (100 mg/kg).

The evaluation of biochemical parameters showed hypertriglyceridemia, hypercholesterolemia and elevated transaminases in hyperlipidemic untreated animals compared to normal controls. The increase of triglycerides (TG) in hyperlipidemic control is due to hepatic triglyceride synthesis induced by fructose [18]; which results hepatic steatosis. Hepatic insulin resistance usually leads to an increase in transaminases [19]. The liver is the storage location and preferred TG synthesis. These TG synthesized by hepatocytes are intended to be incorporated into lipoproteins primarily in VLDL (Very -Low - Density Lipoproteins) and then be excreted in the blood. They are transported by blood to the peripheral tissues, particularly adipose tissue, in which they

are stored. When dietary lipid rises, there are an accumulation of triglycerides; the liver becomes fatty [18]. The administration of fractions of the supernatant and ethyl acetate as metformin significantly reduced hypertriglyceridemia and hypercholesterolemia induced by fructose lard diet; values of the treated groups were similar to that of normal control group. In addition, fractions of *P. alba* lowered the AST compared to hyperlipidemic untreated group. The present results show that the extract of *P. alba* ethyl acetate and supernatant fraction prevents weight gain, accumulation of intra-abdominal fat and hyperglycemia in mice fed with lard- fructose mixture.

Conclusion

The current study indicates that the *Plumeria alba* fractions in mice fed a high-fructose fat diet can prevent the development of hyperglycemia and hyperlipidemia. It suggests that anti diabetic compound of *P. alba* can be concentrated in the two studied fractions. Further phytochemical investigations are useful to identified active compounds of *P. alba* ethyl acetate and supernatant fractions. The present study also provides additional evidence in support the use of this plant in the treatment of diabetes mellitus traditionally in Togo.

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