ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Preventive Effect of *Milicia excelsa* (Moraceae) Aqueous Extract on Dexamethasone Induced Insulin Resistance in Rat.

Paul Désiré Djomeni Dzeufiet[a]*, Marie-Claire Tchamadeu[ab], Danielle Claude Bilanda[a], Yolande Sandrine Mengue Ngadena[a], Mireille Kameni Poumeni[a], David Nana[a], Théophile Dimo[a], Pierre Kamtchouing[a]

[a] Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon
[b] Department of Biological Sciences, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

ABSTRACT

We investigated the preventive effect of the aqueous extract of *Milicia excelsa* (*M. excelsa*) on insulin resistance induced by dexamethasone in rat. Insulin resistance was induced by intraperitoneal injection of dexamethasone (200 µg/kg) during 21 days. The animals were divided into 5 groups 6 in each. Treated groups received concomitantly with dexamethasone, the plant extract (50 and 100 mg/kg) or metformin (200 mg/kg). At the end of the treatment, blood glucose, plasma insulin, aspartate and alanine transaminase (AST and ALT), urea, creatinin and lipid profile were estimated. HOMA-IR index was calculated as a marker of insulin resistance. *M. excelsa* significantly prevented the body weight lost, the increase in blood glucose, insulin, triglyceride, total-cholesterol and ALT. The index of insulin resistance was significantly reduced ($P<0.001$) by 57.53 and 66.64% with *M. excelsa* at the doses of 50 and 100 mg/kg, respectively as compared to dexamethasone. At the same doses, the plant extract significantly prevented the rise of blood glucose 2 hours after glucose load by 15.43 and 18.67 %, respectively, as compared to dexamethasone. These data indicated that *M. excelsa* aqueous extract might prevent the development of dexamethasone-induced insulin resistance and related abnormalities in rats.

Keywords: *Milicia excelsa*, dexamethasone, insulin resistance, rats.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Dexamethasone is a glucocorticoid which is known to induce insulin resistance when it is administered during excess condition [1]. Insulin resistance is defined as the reduced ability of tissues or cells to respond to normal levels of insulin, leading therefore to metabolic disorder and type 2 diabetes. It's a chronic disease related to defective insulin secretion, to insulin resistance. The World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes and this number is likely to more than double by 2030 [2]. The high prevalence of diabetes as well as its long-term complications contributes to increase mortality and morbidity. The need of prevention is therefore for high importance. Many large studies have investigated the prevention of type 2 diabetes in people at high risk of the condition. In fact, prevention of type 2 diabetes has a positive effect on overall wellbeing and quality of life, meaning that more focus should be given to prevention interventions for this condition [3]. This is usually done with changes in diet, physical activity levels and oral antidiabetic drug like metformin [4]. However, alternative therapies are also used, including plant products [5]. Medicinal plants are also traditionally used in many countries to control diabetes. Approximatively, 80% of rural African communities still used phytotherapy to control or treat diabetes mellitus. The use of these plants is based on the belief that they have low toxicity and cost less than the semi-synthetics or synthetics.

Milicia excelsa (Moraceae) is a big tree up to 50 m high and 10 m in girth, growing in dense forest and forest galleries as well as savannah regions. In Cameroon traditional medicine, the leaves are used in treating splenomegaly and otitis, while the bark is used in the treatment of irregular menstruation, constipation and diabetes [6, 7].

Thus, objective of the present work was to investigate the preventive effect of *Milicia excelsa* on dexamethasone induced insulin resistance in rats.

MATERIAL AND METHODS

Plant material and extraction

The barks of *Milicia excelsa* were harvested in Yaounde, Cameroon, in August 2012. It was identified at the Cameroon National Herbarium, Yaounde, Cameroon, by comparison to the voucher specimen N° 57069HNC.

The air-dried barks of *Milicia excels*a were powdered and 1 kg mixed with 2 L of distilled water and brought to ebullition for 10 min. The decoction obtained was filter with a filter paper (Whatman N°3) and dried into an oven at 45°C to obtain 95g of powder (yield of 9.5%). This extract was solubilized in distilled water prior to administering to the experimental animals.

Qualitative phytochemical determination tests

Phytochemical determination of the aqueous extract of *M. excelsa* were tested using various reagents: Mayer and Dragendoff's reagents for alkaloids, magnesium chip and HCl

for flavonoids, $FeCl_3$ for tannin, frothing test for saponin, diethyl ether, sulfuric acid and anhydride acetic for steroids, ether-chloroform and NaOH for anthraquinones, NaCl, and Fehling's solutions for glycoside, $FeCl_3$ and $K_3Fe(CN)_6$ for phenols and polyphenols [8].

Animals

Male Wistar albino rats of 3 months old, weighing 220-250 g, were used for our experiment. They were kept and maintained under standard laboratory conditions of temperature, humidity, with a natural day/night light cycle and allowed free access to rat chow and water. Fasting rats were deprived of food for at least 14 h but not water. Fasted normoglycemic rats with blood glucose levels of 72-80 mg/dL were used in our experiments. Prior authorization for the use of laboratory animals was obtained from the Cameroon National Ethics Committee (Ref. N°. FWIRB 00001954).

Animal grouping, Induction of insulin resistance and treatment

Dexamethasone (Sigma, St. Louis, MO., USA) used in this experiment was previously dissolved in 10% ethanol in normal saline as prescribed by the supplier. A total of 30 rats were used. They were divided into five groups of six rats each:

- Group 1, normal control (untreated) rats, receiving by gavage distilled water (10 mL/kg) and intraperitoneal injection (1 mL/kg) of 10% ethanol in normal saline (vehicle);
- Group 2, dexamethasone insulin resistant control rats, receiving by gavage distilled water (10 mL/kg) and intraperitoneal injection of dexamethasone (200 µg/kg);
- Group 3, rats receiving by gavage metformin (200 mg/kg) and intraperitoneal injection of dexamethasone (200 µg/kg);
- Group 4, rats receiving by gavage the aqueous extract of *M. excelsa* (50 mg/kg) and intraperitoneal injection of dexamethasone (200 µg/kg);
- Group 5, rats receiving by gavage the aqueous extract of *M. excelsa* (100 mg/kg) and intraperitoneal injection of dexamethasone (200 µg/kg).

The selection of the doses of the plant extract used was based on the posology of the tradi-practitioners. The experimentation was carried out for 21 days, during which treatments were given every day between 8 to 9 AM.

Oral glucose tolerance test (OGTT)

At the end of the treatment period, rats were submitted to OGTT by oral administration of glucose (5 g/kg). They were deprived of food for at least 14 h before and during the experiment, but were allowed free access to tap water. Blood was withdrawn from the tail tip before glucose solution administration (time 0) and at 30, 60, 90 and 120 min after glucose administration. Blood glucose content was measured using a commercial glucometer (Acku-check, Roche, Germany).

Biochemical analysis

At the end of the experimental period, the animals were sacrificed by decapitation and the blood samples were collected. The samples were centrifugated and the plasma obtained was frozen at -20°C for biochemical analysis. Plasma triglyceride, total cholesterol, HDL-cholesterol, bilirubin, urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using commercial diagnostic kits, Fortress Diagnostics, UK.

Determination of insulin levels and Index of insulin resistance

Serum insulin was determined using the rat insulin ELISA kit (Crystal Chem. Inc., USA) by ELISA reader.

The index of insulin resistance was estimated by the homeostasis model assessment (HOMA) and calculated using relationships between the blood glucose and insulin levels according to the following formula: HOMA-IR index = [fasting glucose (mg/dL) x fasting insulin (mU/mL)]/ 405) [9].

Conversion factor: Insulin (1U/L = 7.174 pM)

Statistical analysis

All the results were expressed as mean ± SEM for 6 animals in each group. Statistical differences between control and treated groups were tested by one way analysis of variance (ANOVA) followed by Dunnett's comparison tests using Stat-Direct-Software. P-values of less than 0.05 were considered to indicate statistical significance

RESULTS

Quantitative phytochemistry

Polyphenol, phenol, flavonoids, saponins, triterpenes, tannins and glycosides were identified whereas alkaloids, steroids and anthraquinones were absent.

Effects of *M. excelsa* on body weight

During the experiment, we recorded a gradual increase in body weight of normal rat, with a percentage of increased of 8.47% as compared to initial value (Figure 1). On the contrary, rats receiving only dexamethasone showed a significant ($P<0.01$) decrease (20.19%) in body weight as compared to the initial value. Concomitant administration of dexamethasone with *M. Excelsa* extract or metformin prevented the lost of body weight induced by dexamethasone.

ISSN: 0975-8585

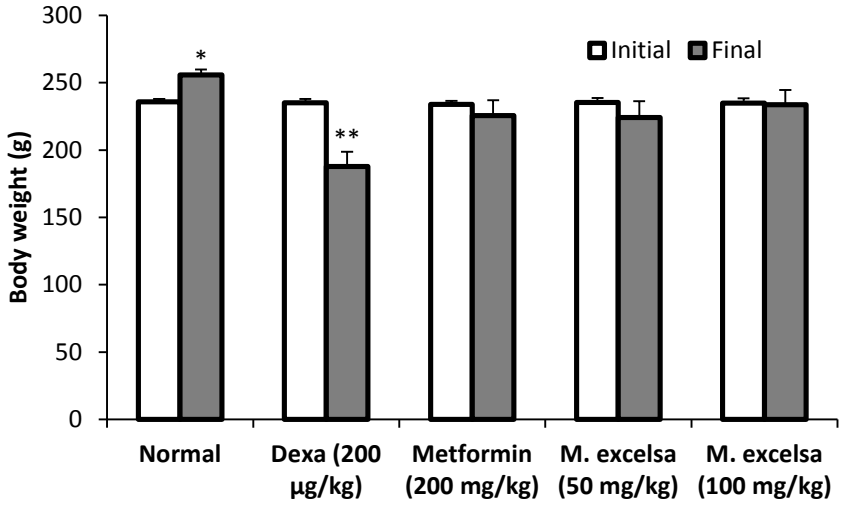

Figure 1: Effect of *M. excelsa* on the body weight of experimental rats.

Each bar represents a mean ± ESM, n= 6, *p<0.05, **p<0.01, compared with initial value. Dexa= dexamethasone.

Effects of *M. excelsa* on blood glucose and insulin levels

The results of the blood glucose variation are shown in figure 2. Intraperitoneal administration of dexamethasone resulted in a significant (P<0.01) increase in blood glucose. As compared to initial value, there was 44.87% increase in blood glucose. *M. Excelsa* aqueous extract as well as metformin prevented the rise in blood glucose observed with dexamethasone alone.

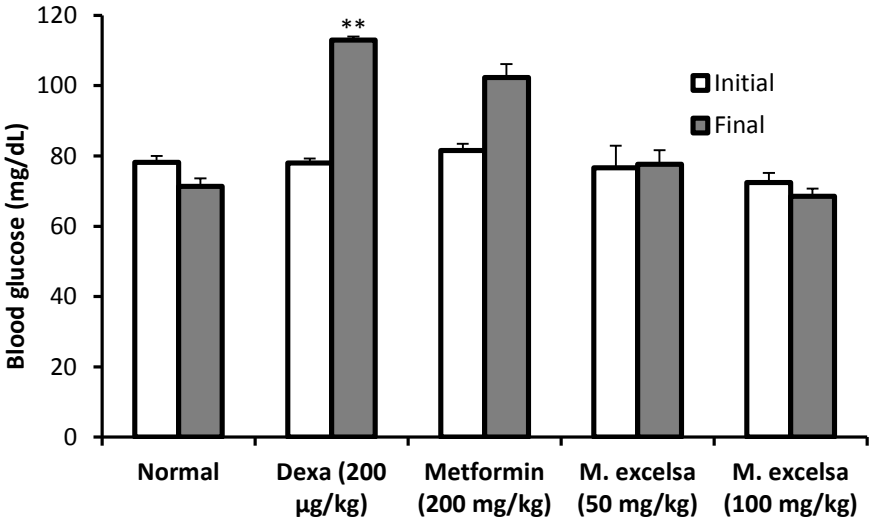

Figure 2: Effect of *M. excelsa* on blood glucose.

Each bar represents a mean ± ESM, n= 6, **p<0.01, compared with initial value. Dexa= dexamethasone.

Blood insulin levels of experimental rats are recorded in figure 3. Rat receiving dexamethasone only exhibited a significant (P<0.001) increase of the blood insulin level (118 %) as compared to initial value. When rats were treated with *M excelsa* or metformin, there was a significant prevention of the rise of insulin level. When compared to dexamethasone rats, there were 34.95, 38.19 and 45.02 % reduction in blood insulin respectively for rat

ISSN: 0975-8585

treated with metformin, *M. Excelsa* at the dose of 50 mg/kg and *M. Excelsa* at the dose of 100 mg/kg.

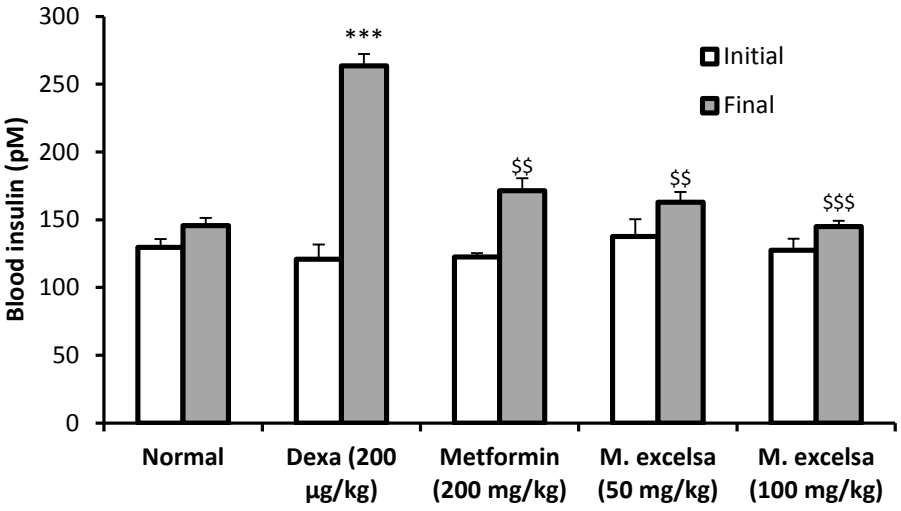

Figure 3: Effect of *M. excelsa* on blood insulin.

Each bar represents a mean ± ESM, n= 6, ***p<0.001, compared with initial value $^{\$\$}$p< 0.01, $^{\$\$\$}$p<0.001, compared with dexamethasone, Dexa= dexamethasone.

Effect of *M. Excelsa* on the homeostasis model assessment index for insulin resistance (HOMA-IR)

Our results showed that administration of dexamethasone significantly (P<0.001) increased the calculated HOMA-IR in rats (Figure 4). There was 186.33% increase in that parameter as compared to normal rat. When rats were treated with *M. excelsa*, we recorded 57.55 and 66.64% reduction in HOMA-IR as compared to dexamethasone rats at the respective doses of 50 and 100 mg/kg. Metformin also produced a significant (P<0.01) reduction of HOMA-IR as compared to dexamethasone rats.

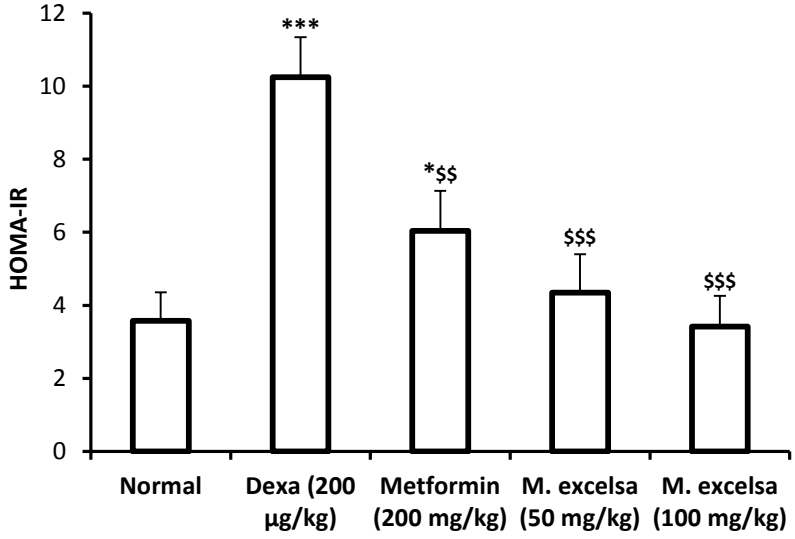

Figure 4: Effect of *M. excelsa* on index of insulinresistance.

Each bar represents a mean ± ESM, n= 6, *P<0.05, ***p<0.001, compared with normal, $^{\$\$}$p< 0.01, $^{\$\$\$}$p<0.001, compared with dexamethasone, Dexa= dexamethasone.

ISSN: 0975-8585

Effect of *M. excelsa* on oral glucose tolerance test (OGTT)

During the OGTT, the blood glucose levels of dexamethasone rats were significantly higher at all considered time as compare to normal rats (Figure 5). Concerning the 120 min blood glucose values, there was no significant difference in treated rat (*M. excelsa* and metformin) as compared to normal rats. When compare with dexamethasone rats, there was a significant decrease of the blood glucose by 15.40, 18.67 and 17.12% respectively for *M. excelsa* at the dose of 50 mg/kg, *M. excelsa* at the dose of 100 mg/kg and metformin.

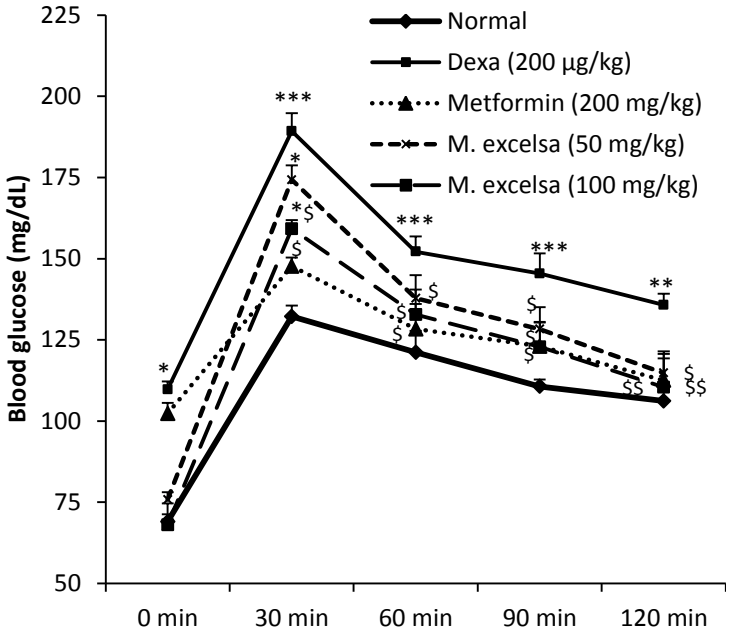

Figure 5: Effect of *M. excelsa* on oral glucose tolerance test.

Each point represents a mean ± ESM, n= 6, *P<0.05, **P<0.01, ***p<0.001, compared with normal rat at the same time point, $^{\$}$p<0.05, $^{\$\$}$p< 0.01, compared with dexamethasone at the same time point, Dexa= dexamethasone.

Effect of *M. excelsa* administration on some biochemical parameters of experimental rats

Studies on other biochemical parameters showed that at the end of treatment, serum lipid levels (triglycerides and total-cholesterol) and serum alanine aninotrasferase (ALT) were significantly increased in dexamethasone rats as compared to normal rats. When rats were treated with the plant extract or metformin, there was a significant reduction of total-cholesterol by 43.09, 53.80 and 31.63 % respectively for *M. excelsa* at the dose of 50 mg/kg, *M. excelsa* at the dose of 100 mg/kg and metformin. At the same doses, the plant extract prevented the increased of triglycerides by 42.87 and 42.45 % as compared to dexamethasone rats. There was no significant change in the levels of bilirubin, urea and creatinin.

ISSN: 0975-8585

Table 1: Effect of *M. excelsa* administration on some biochemical parameters of experimental rats

	Normal	Dexa (200 µg/kg)	Metformin (200 mg/kg)	*M. excelsa* (50 mg/kg)	*M. excelsa* (100 mg/kg)
Triglyceride (mg/dL)	33.92±1.94	76.29±8.47**	49.04±5.49	43.58±0.46$^{\$\$}$	43.90±1.70$^{\$\$}$
Total cholesterol (mg/dL)	52.62±4.27	71.56±1.63*	48.92±4.11$^{\$}$	40.72±0.49$^{\$}$	33.06±0.64$^{\$}$
HDL-cholesterol (mg/dL)	20.94±1.54	14.65±1.76	19.18±1.88	17.24±0.22	15.02±0.21
ALT (U/L)	48.86±5.71	71.74±4.03*	50.28±6.00$^{\$}$	68.08±1.80*	56.74±2.95$^{\$}$
AST (U/L)	194.14±23.04	201.48±8.60	199.5±19.45	179.12±4.79$^{\$}$	174.44±6.81$^{\$}$
Bilirubin (mg/dL)	0.13±0.02	0.19±0.03	0.16±0.02	0.13±0.01	0.13±0.02
Urea (mg/dL)	39.20 ±1.54	43.64±1.82	37.54±4.10	41.76±0.63	36.54±0.89
Creatinin (mg/dL)	0.64±0.01	0.63±0.03	0.57±0.03	0.54±0.01	0.50±0.00

Values are expressed as mean ± ESM, n= 6, *p<0.05, **p<0.01, ***p<0.001, compared with normal rats $^{\$}$p< 0.05, $^{\$\$}$p<0.01, compared with dexamethasone. Dexa= dexamethasone.

DISCUSSION

Dexamethasone has been frequently used to induce insulin resistance in experimental animals [1]. The present study has shown that dexamethasone induced body weight lost, increased blood glucose, insulin, triglycerides, total-cholesterol, ALT and insulin resistance index. Those results strongly suggest the induction of insulin resistance *in vivo*. *Milicia excelsa* aqueous extract was capable to prevent the installation of insulin resistance due to injection of dexamethasone.

The results from this study revealed significant (P<0.01) loss of weight of dexamethasone rats compared to normal animals. This may be due to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein. Treatment with the plant extract and metformin prevented the body weight loss. *M. excelsa* might act by potentiating insulin action in muscles and or adipose tissue. In fact, insulin is known to stimulate proteogenesis and to inhibit proteolysis. Our results are similar to those of Das et al., [10] who showed that *Azadirachta indica* aqueous extract stimulate weight gain after 21 days administration.

Dexamethasone significantly increased the fasting blood glucose of rats. This may be due to the fact that dexamethasone induced PI_3-K deactivation at the level of muscles and adipose tissue, leading to a reduction of glucose uptake. In fact, PI_3-K is essential for glucose transport into cells, since it stimulates the transloation of Glut 4 from cytosol to the plasma membrane [11]. This can also explained the significant increase of blood insulin and therefore the high index of insulin resistance expressed by HOMA-IR. *Milicia excelsa* significantly and dose dependently prevented the rise in blood glucose, blood insulin and insulin resistance. Furthermore, from oral glucose tolerance test, it could be concluded that the plant extract showed an improvement in glucose tolerance as compared to dexamethasone untreated rats. The activity of the aqueous extract of *M. excelsa* may be related to the presence of bioactive compounds such as polyphenols, flavonoids, triterpenes, glycosides, which have been reported to significantly reduce blood glucose levels. Some triterpenes from *Gymnema sylvestre*, flavonoids from *Pterocarpus marsupium*, glycosides isolated from *Ceiba pendandra* have been shown to significantly reduce blood

glucose levels [12, 13]. Possible mechanisms of action of these compounds include insulin-like activities [14].

Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart disease which are diabetes complications [15, 16]. In the present study, dexamethasone significantly increased the level of total cholesterol and triglycerides as compared to normal rats. This may be the consequence of insulin resistance observed in this model. *M. excelsa* aqueous extract significantly prevented the rise in serum total cholesterol and triglycerides in treated rats. Thus, it is reasonable to conclude that *M. excelsa* could modulate blood lipid abnormalities.
ALT level was also increased by dexamethasone administration, suggesting a deleterious action on liver function. In fact, dexamethasone is known to enhance mRNA of PPAR-α expression of the liver, leading to excess glucose release and then to glucotoxicity [17, 18]. Glucotoxycity is characterized by the production of free radical which induced lipid peroxydation and then leakage of ALT into blood stream. The normalization of transaminases levels under *M. excelsa* treatment, suggest an antioxidant property, due at least in part to the presence of flavonoids and polyphenols in the aqueous extract.

The preventive activity of *M. excelsa* was compared with metformin, a standard antidiabetic drug. Since the results of the present study shows similarity in the plant extract and metformin, it may be suggested that the mechanism of action of *M. excelsa* may be similar to metformin action.

In conclusion, *Milicia excelsa* aqueous extract can prevent the resistance in insulin action in dexamethasone rat model. This justifies the traditional used of the plant for the management of type 2 diabetes and open a way for investigation for new antidiabetic drug.

REFERENCES

[1] Gounarides JS, Korach-Andre M, Killary K, Argentieri G, Turner O, Laurent D. Endocrinol 2008; 149: 758–66.
[2] WHO 2009 http://www.who.int/mediacentre/factsheets/fs312/en/index.html.
[3] Nikki JM, Sara A, Aliceon B, Melanie D, Mike JS. British J Diab Vascul Disea 2011;11(6):308-12.
[4] Yael W. Diabetes Care. 2012; 35: 663-65.
[5] Sabo CE, Rush MS, Temple LL. Diabetes Educ 1999 ; 25: 945-56.
[6] Adjanohoun JE, Aboubakar N, Dramane K, et al. (CSTR/OUA or OAU/STRC), 1996; 641.
[7] Ndenecho EN. AJPP 2009; 3(3): 78-86.
[8] Odebiyi O, Sofowora EA. L Loydia 1978; 41: 41-234.
[9] Matthews DR, Hosker JR, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Diabetologia 1985; 28: 412–19
[10] Das RA, Mostofa M, Hoque EM, Das S, Sarkar KA. Bengl J Veter Med 2010; 8(1): 75-80.
[11] Kahn CR, Folli F. Hormon Resh 2004; 39(3): 93-101.
[12] Grover JK, Yadav S, Sats V. J Ethnopharmacol 2008; 81: 81-100.
[13] Dzeufiet DPD, Tedong L, DimoT, Assongalem EA, Sokeng DS, Kamtchouing P. Indian J

Pharmacol 2006; 38(3) 194-7.
[14] Sakurai T, Nishimuta T, Otake N, Xinsheg Y, Abe K, Zeida M, Nagasawa H, Sakuda S. Bioorganic Med Chem Letters 2002; 12 (5): 807-10.
[15] Dzeufiet DPD, Ohandja YD, Tedong L, DimoT, Assongalem EA, Sokeng DS, Kamtchouing P. Afr. J. Trad. CAM 2007; 4(1) 47-54.
[16] Dzeufiet DPD, Nguelefack TB, Azay J, Kamtchouing P, Cros G, Dimo T. RJPBCS 2014; 5(2): 707-15.
[17] Bernal-Mizrachi C, Weng S, Feng C, Fink BN, Knutsen RH, Leone TC, Coleman T, Mecham RP, Kelly DP, Semenkovich C. Nature Medicine 2003; 1069-75.
[18] Valko M, Leibfritz D, Moncol J, Cronin M, Mazur M, Telser J. Int J Biochem Cell Biol 2007; 39: 44-84.