International Journal of Phytomedicine 9 (2017) 267-274
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Hibiscus noldea (Malvaceae) aqueous extract prevents insulin resistance and protects pancreatic islets from dexamethasone damages in rat.

Ngueguim Tsofack Florence[1*], Djientcheu Tientcheu Jean Philippe[1], Donfack Jean Hubert[2], Gounoue Kamkumo Raceline[1] , Dzeufiet Djomeni Paul Desire[1] , Pierre kamtchouing[1] Theophile Dimo[1]

***Corresponding author:**

Ngueguim Tsofack Florence

[1]Department of Animal Biology and Physiology, Faculty of Sciences, , University of Yaoundé I, P.O.Box 812, Yaoundé, Cameroon.
[2]Department of Biomedical Sciences, Faculty of Science, University of Dschang , P.O. Box 67, Dschang, Cameroon.

Abstract

Hibiscus noldea leaves-stems aqueous extract is used in Cameroonian traditional medecine to manage diabetes. To investigate the preventive effect of *Hibiscus noldea* aqueous extract on dexamethasone-induced insulin resistance, the animals received one of the following treatments: distilled water (10 mL/kg), metformine (200 mg/kg), or *H. noldea* (100 or 200 mg/kg) concomitantly with dexamethasone (0.5 mg/kg, ip) for ten days. Body weight was evaluated daily and blood glucose levels were measured. At the end of experiment, insulin sensitivity test was performed and lipid profile, transaminases Aspartate amino transferase, Alan in amino transferase, malondialdehyde, superoxide dismutase, catalase, and reduced glutathione were evaluated. Histological analysis of the liver was investigated to estimate glycogen content using Periodic Acid Schiff coloration and histomorphometry of pancreatic islets area was performed.

The administration of dexamethasone during ten days induced body weight loss, hyperglycaemia, insulin resistance, an imbalance in lipid profile, an increase in transaminases and oxidative stress. Dexamethasone treatment also induced an increase in the pancreatic islets area and depletion in the levels of hepatic glycogen. Concomitant administration of dexamethasone and the aqueous plant extract prevented the rise in blood glucose levels, reduced insulin resistance, improved lipid profile and oxidative status. The aqueous extract of *H. noldea* prevented the use of glycogen storage and the increase in pancreatic islet area in dose dependent manner.

Conclusion: The stem leaves aqueous extract from *Hibiscus noldea* have the ability to reduce insulinresistance via its antihyperglycaemic, hypolipidemic and antioxidant activities. These results justify the use of this extract in the management of diabetic state.

Keywords: dexamethasone, insulin resistance, hyperglycaemia, pancreatic islets area, *Hibiscus noldea*

Introduction

Insulin resistance is a pathology characterized by hyperinsulinemia, hyperglycaemia or both which can be associated with visceral obesity, dislipidemia and high blood pressure. Many factors can induce insulin resistance among which glucocorticoïds as dexamethasone. Dexamethasone is currently used in some inflammatory pathology and auto-immune diseases [1]. It is well known that, excess glucocorticoid induces insulin resistance which is related to the developpement of type 2 diabetes [2,3]. The mechanism by which glucocorticoid induces hyperglycaemia is the stimulation of some key enzymes involved in gluconeogenesis. Moreover recently, high doses of dexamethasone have shown to induce ß cell proliferation in pancreatic islets [4] contributing to hyperinsulinemia. Although severals works have been done in the management of diabetes mellitus using medicinal plants, researches still going on around the world to, find new drugs with minor adverse effects. *Hibiscus noldea* is used against abortion and headache in Cameroonian traditional medicine [5,6]. In addition, the mixture of leaves and stem of this plant in the form of decoction is used by “Bamileké” peoples to manage diabetes mellitus [7]. Considering that insulin resistance is one of the factors involved in the diabetes physiopathology, thus the reduction of insulin resistance could contribute to the management of diabetes. Therefore, the present study aims to reveal the possible role of *Hibiscus noldea* extracts in the prevention of dexamethasone-induced insulin resistance.

DOI:10.5138/09750185.2036

This article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use and redistribution provided that the original author and source are credited.

Material and Methods

The fresh stem leaves of *H. noldeae* were harvested at ODJA (Centre Region of Cameroon) in May 2015 and authentication was done by comparing the sample Nº 9977 of the National Herbarium of Cameroon. The samples were dried in the shade at room temperature and were crushed into fine powder. The powder (500g) was boiled in 7.5 L of tap water for 10 minutes according to the protocol of traditional healer. After filtration of the mixture, the filtrate collected was lyophilized at the Institute of Medicinal and Medical Plants (IMMP). The yield of the extract was 6.43% (W/W).

Experimental animals

The experiments were performed on male albinos Wistar rats aged approximately of three months and weighting between 250g-270g. The animals were raised in the animal house of the Faculty of Science, University of Yaoundé I. They were housed together (5 rats per cage), maintained at room temperature (22 ± 2 C) with adequate ventilation and free access to tap water and food. These studies were conducted with the approval of the Cameroon National Ethical Committee (Ref nº.FW-IRB00001954).

Experimental design

Thirty rats were divided into five groups of six rats each as follow:
Normal control rats receiving distilled water and NaCl (0.9%); negative control group receiving dexamethasone 0.5 mg/kg and distilled water (Dexa 0.5 + DW), three groups receiving dexamethasone 0.5 mg/kg and metformin 200 mg/kg per os or *H. noldeae* (Dexamethasone 0.5 mg/kg and extract 100 mg/kg or 200 mg/kg)
Animals received these daily treatments during 10 days. At the end of the experimental period, all animals were subjected to insulin sensitivity test following our previous protocol [8]. Briefly, after 12 hours of fasting, the glycaemia was evaluated (0 h) and animals received 2 UI/kg of insulin and blood was obtained from tail at 10, 20, 30 and 60 min after insulin injection, then serum glucose was measured.

Blood analysis

Animals were sacrificed under anaesthesia and arterial blood was collected and centrifuged at 3000 g at 4 C for 10 min to obtain serum (stored at 20 C until analysis) for biochemical analysis (glucose, ALAT, ASAT, creatinine, triglycerides, total cholesterol, HDL chlolesterol, LDL chlolesterol and atherogen index determined by the ratio of total cholesterol/HDLcholesterol) [9]. These parameters were quantified spectrophotometrically according to the commercial instructions for the kits.

Homogenate analysis

Homogenates (20%) of liver, heart and kidney samples were prepared in Tris–KCl buffer (pH 7.4). Organs were crushed and then the mixture was centrifuged at 3000 g at 4°C for 20 min. The supernatant was collected and stored at 20 C until tissue analysis of SOD, malondialdehyde, glutathione and catalase.
Histomorphometry of pancreatic islets
Histomorphometry of pancreatic islet size was performed after heamatoxylin-eosin staining. The area of pancreatic islet was measured using software of area measurement (ImageJ, version 1.49)

Determination of glycogen content

Liver samples were fixed in 10% buffered par formaldehyde, dehydrated in graded alcohol series. They were embedded in paraffin and a thickness of 5 µm sections was made using a microtome (Reichert-Jung 2030). Glycogen analysis was assessed by periodic acid Schiff (PAS) staining method. Photograph of the sections were taken using a digital camera for microscope (DCM 35:350 K Pixels, USB 2.0) aided with appropriate filters.

Statistic analysis

All data are expressed as mean ± standard error mean. Statistical significance was determined by one way analysis of variance followed by the Tukey post-test using Graph pad Prism version 5.03.0. Differences were considered significant at $p < 0.05$.

Results

Effects of *Hibiscus noldea* on body weight

Daily administration of dexamethasone during 10 days induced a significant decrease ($p< 0.01$) of body weight as compared to normal control. However the plant extract at the dose of 200 mg/kg significantly prevented the decrease of body weight as compared to negative control; even if the body weight was still low as compared to the normal control. The extract at the dose of 100 mg/kg failed to prevent dexamethasose-induced body weight loss.

Effects of *Hibiscus noldea* on insulin sensitivity

Animal receiving dexamethasone showed a significant reduction ($p< 0.01$) in insulin sensitivity 20 minutes following the injection of insulin. However,insulin significantly failed to reduce blood glucose levels of these animals (Figure-2) 60 min later as compared to normal control. Simultaneous administration of the plant extract or metformine with dexamethasone decreased blood glucose levels as compared to their initial value. Only the plant extract at the dose

of 200 mg/kg prevented the decrease in insulin sensitivity illustrated by a significant decrease in glycaemia when compared to the negative control (Dex 0.5 + DW).

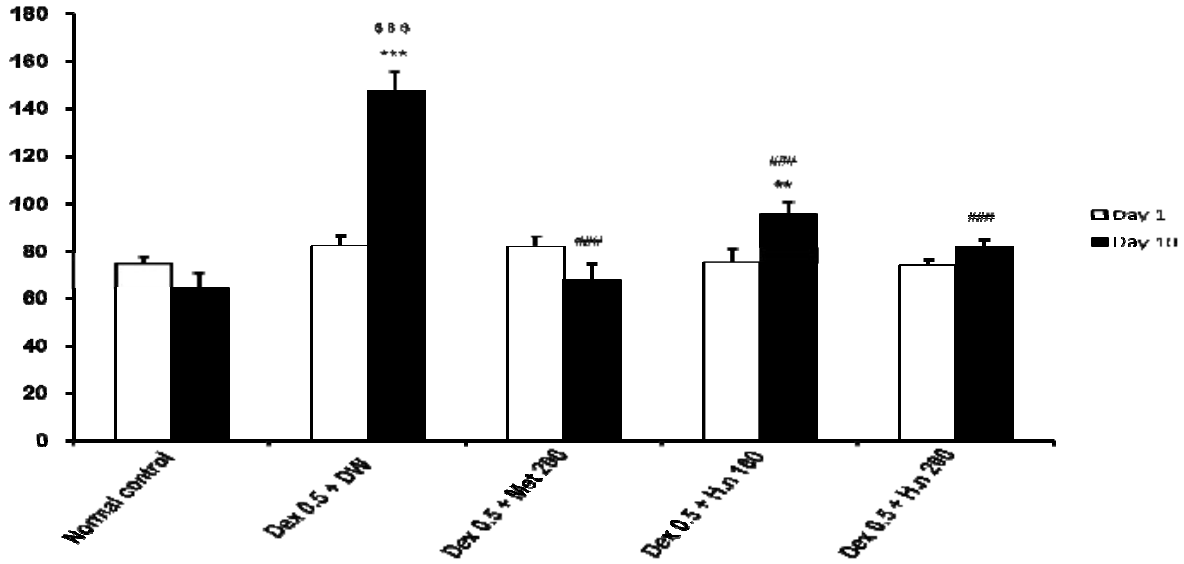

Figure-2: Effects of the aqueous extract of *Hibiscus noldea* on Dexamethasone induced-hyperglycaemia. Each bar represents Mean ±SEM, n=6. **p<0.01, ***p<0.001, significantly different from normal control at each time.###p<0.001 significantly different from group Dex 0.5 + DW.$$$p<0.001, significantly different from day 1.

Effects of *Hibiscus noldea* on blood glucose levels

Figure-3 depicts the effect of the aqueous extract of *Hibiscus* noldea on blood glucose variation in dexamethasone-induced hyperglycemia. The administration of dexamethasone for 10 days induced a significant increase in blood glucose levels when compared to the initial value. Concomittant administration of dexamethasone and the plant extract during ten days prevented the increase of blood glucose levels. The decrease (p< 0.01) was 35.20% and 44.61% respectively at the doses of 100m/kg and 200 mg/kg in comparison to negative control. Metformine in the same conditions prevented the increase in glycaemia by 54.07%. The plant extract and metformin at the dose of 200 mg/kg maintained blood glucose levels around normal value.

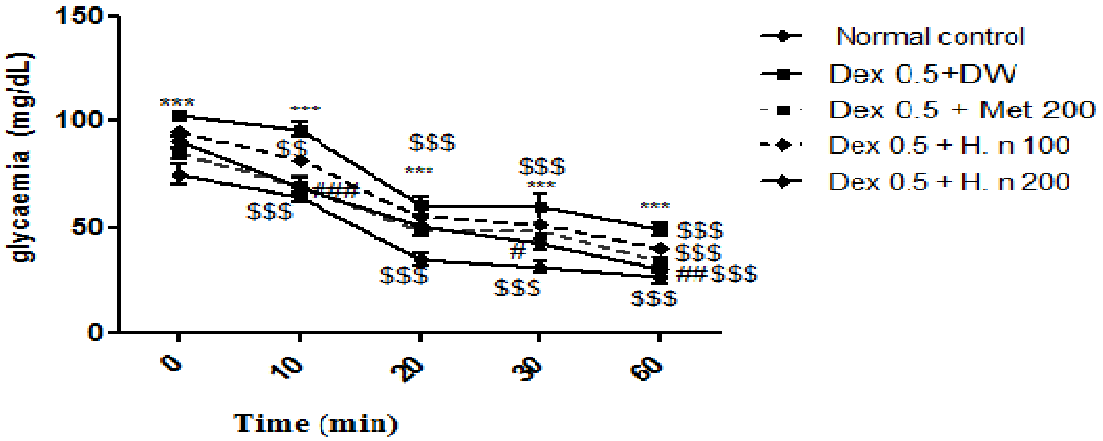

Figure-3: Effects of the aqueous extract of *Hibiscus noldea* on insulin sensitivity. Each point represents Mean ± SEM, n=6, ***p<0.001, significantly different from normal control, #p<0.05, ##p<0.01, ###p<0.001 significantly different from group Dex 0.5 + DW, $p<0.05, $$p<0.01, significantly different from initial value.

Effects of *Hibiscus noldea* on lipid profile and hepatic function

Table 1 represents the effects of aqueous extract of *Hibiscus noldea* on lipid profile and hepatic function after ten days of co-administration of dexamethasone with different treatments. The administration of dexamethasone for 10 days induced a significant increase in the level of total cholesterol, triglycerides, LDL-cholesterol and atherogenic index, while HDL cholesterol levels significantly decrease. Co-administration of dexamethasone with the *H. noldea* aqueous extract prevented the increase in total cholesterol, LDL cholesterol and atherogenic index respectively at the dose of 100 mg/kg ($p< 0.01$) and 200 mg/kg ($p< 0.001$). The plant extract at all doses failed to affect triglycerides and HDL cholesterol levels. Dexamethasone administration for ten days induced a significant increase in the ALT and AST activities as compared to the normal control. Concomitant administration of the plant extract with dexamethasone produced a significant decrease ($p< 0.01$) in ALT activities at all doses. This treatment also prevented the increase in AST activity at the dose of 200 mg/kg but failed at the dose of 100 mg/kg. Metformine used as reference drug exhibited a significant decrease in ALT and AST activities when compared to the control.

Table-1 : Effects of the aqueous extract of *Hibiscus noldea* on lipid profile and some parameters of hepatic function.

Parameters	Normal control	Dex 0.5 + DW	Dex 0.5 + Met 200	Dex 0.5 + H. n 100	Dex 0.5 + H. n 200
Total cholesterol (mg/dL)	96.96±13.23	195.02±10.88***	110.98±10.95###	121.86±16.38###	112.55±10.33###
Triglycerides (mg/dL)	39.66±2.18	88.19±11.26**	42.84± 8.31##	61.70±9.48	73.93± 8.65
LDL Cholesterol (mg/dL)	38.13±15.66	140.73±11.90***	37.66±13.65###	53.35±12.07##	48.89±15.72###
HDL Cholesterol (mg/dL)	50.89±3.59	36.64±4.28	64.74 ± 7.56#	56.17±7.53	48.86±6.09
Atherogenic index	2.02±0.40	5.72 ± 0.74***	1.82±0.29###	2.19±0.17 ###	2.53±0.42 ###
ALT (UI /L)	38.82±7.82	126 ± 2.99***	58.83±9.34 ###	39.32±3.73 ###	47.87±4.63###
AST (UI /L)	115.12±11.43	170.00 ± 15.17*	105.43 ± 7.12#	119.94±15.51	94.76±13.49##

Each value represents Mean ±SEM, n=6. *p<0.05, **p<0.01, ***p<0.001, significantly different from normal control,#p<0.05, ##p<0.01, ###p<0.001 significantly different from Dex 0.5 + DW group.

Effects of *Hibiscus noldea* on pancreatic islets area

After ten days administration of different treatments, histomorphometry of pancreatic islets of the various groups was performed (representative image in Figure.4A).The quantification of pancreatic islets area shows that, daily administration of dexamethasone (0.5 mg/kg) for ten days resulted in a significant increase (45.52%, $p<0.05$) in pancreatic islets area as compared to the normal control (Figure.4B). Simultaneous administration of the plant extract at the dose of 100 mg/kg and 200 mg/kg with dexamethasone prevented the increase in pancreatic islets area respectively by 28.30% ($p< 0.05$) and 37.75% ($p< 0.01$) in comparison with Dexa 0.5 + Dw group. Metformin administered in the same condition as the plant extract significantly prevented the increase in pancreatic islets area by 33.54% ($p< 0.05$).

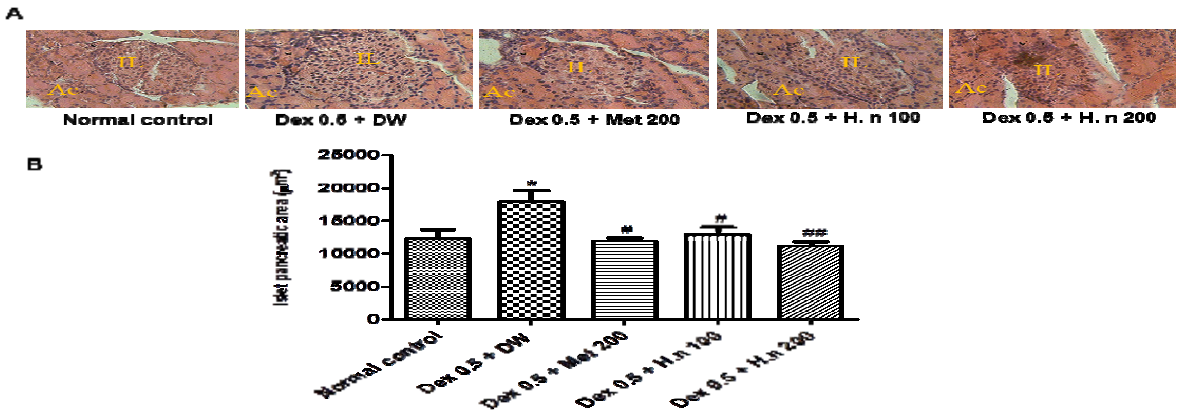

Figure-4 : Effects of the aqueous extract of *Hibiscus noldea* on islet pancreatic area (H-E X400).Each bar represents Mean ±SEM, *p<0.05, significantly different from normal control, ##p<0.01, ###p<0.001 significantly different from group Dex 0.5 + DW. Ac: acinus, IL: islet

Effects of *Hibiscus noldea* on some oxidative stress markers

The effect of the aqueous extract of *Hibiscus noldea* on dexamethasone-induced hyperglycaemia associated to oxidative stress is represented in Figure-5. It was observed that administration of dexamethasone during ten days provoked an increase in MDA levels in the liver (p> 0.05) kidney (p< 0.01) and heart (p< 0.001) (Figure-5A). Dexamethasone administration induced a non-significant reduction of GSH levels in these organs (Figure- 5B). However the catalase activity was significant reduced in the heart (Fig 5C) and SOD activity was decrease (p< 0.05) in the liver and kidney (Figure-5D). It was observed a reduction of catalase activity in the liver, kidney and a decrease of SOD activity in the kidney whenever non significant. Simultaneous administration of dexamethasone and the plant extract at different doses significantly reduced MDA levels and prevented the decrease in both catalase and SOD activities in such organs as compared to the control.

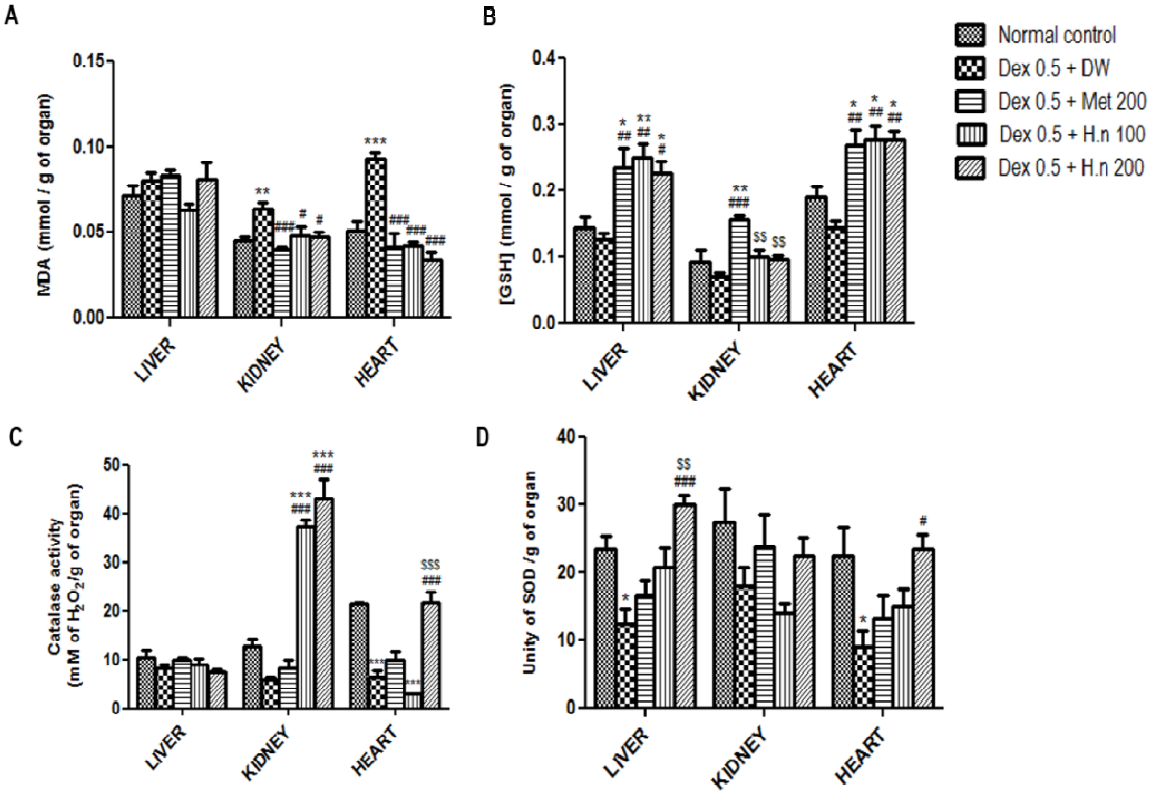

Figure-5 : Effects of the aqueous extract of *Hibiscus noldea* on MDA (A) GSH (B) catalase (C) and SOD(D).*p<0.05, **p<0.01, ***p<0.001, significantly different from normal control, #p<0.05, ##p<0.01, ###p<0.001 significantly different from group Dex 0.5 + DW.$$p<0.01, significantly different from group Dex 0.5 + Met200

Effects of *Hibiscus noldea* on hepatic glycogen content

Hepatic glycogen content was evaluated using Periodic Acid Schiff coloration. The liver glycogen was declined in dexamethasone induced-hyperglycemic rats (Figure-6). Micrography A (normal control) shows the presence of glycogen indicating the high intense staining as compared to micrography B (Normal control +salivary amylase) where the staining disappear with the presence of salivary amylase. As compared to normal control, there was a slight staining in the liver of animals treated with dexamethasone (Micrography C). However the plant extract at the doses of 100 mg/kg (Micrography E) and 200 mg/kg (Micrography F) and even the metformine (Micrography D) prevented the depletion in the liver glycogen characterized by the intense staining. Animal treated with the dose of 200 mg/kg expressed strong staining as compared to negative control.

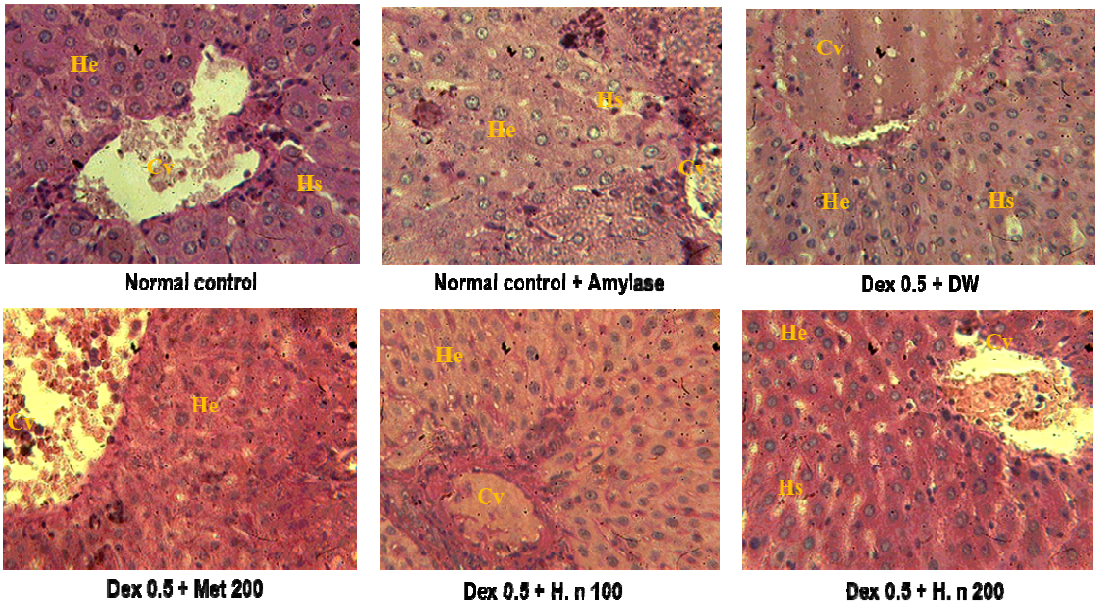

Figure-6: Effects of the aqueous extract of *Hibiscus noldea* on liver glycogen (Periodic Acid Schiff staining X400). (A): Normal control (B):Normal control +salivary amylase; (C):Dex 0.5 + DW; (D):Dexa 0.5 + Met 200 mg/kg, (E): Dexa 0.5 + H. n 100mg/kg; Dexa 0.5 + H. n 200 mg/kg. He: hepatocyte, Cv: centro-lobular vein, Hs: hair sinusoid.

Discussion

In this study, we found out that, daily administration of dexamethasone at the dose of 0.5 mg/kg during ten days, induced body weight loss. Several mechanisms are involved in corticosteroid-induced body weight loss among which the increase in protein catabolism and the decrease in the synthesis of proteins [10] via the reduction of protein kinase B phosphorylation [11]. Concomitant administration of dexamethasone and the plant extract did not prevent the body weight loss except a slight slowdown of body weight loss observed at the dose of 200 mg/kg. These results suggest a slight effect of the plant extract on dexamethasone-induced body weight loss and let us think that, in long term treatment, the plant extract could reverse body weight loss.This assertion needs to be verified. It is well known that insulin resistant is a preponderant factor in the development of diabetes [12]. In this study, the administration of dexamethasone induced hyperglycemia and insulinresistance. Alterations in glucose regulation could be due to the alteration of the binding of insulin to its receptor and/or the suppression of insulin receptor substrate or desactivation of phosphatidylinositol 3-kinase [13] contributing to the reduction of insulin cellular response [14,15]. In addition dexamethasone increases hepatic glucose via the stimulation of the key enzymes of gluconeogenesis and glycogenolysis resulting to hyperglycaemia. Glycogenolysis is confirmed in this study by the decrease in the intensity of coloration of liver tissue, attesting the reduction in glycogen storage. Dexamethasone administration contributes to hyperinsulinemia due to the increase in secretory function of pancreatic cells illustrated in this study by the increase in pancreatic islet size. This phenomenon is an adaptation of pancreatic cells to insulinresistance. The administration of the aqueous extract of *H. noldea* prevented the increase in blood glucose thus reducing insulin resistance and consequently the reduction of pancreatic islets area and glycogenolylsis. This finding suggests that, the extract is able to counteract the effects of dexamethasone at cellular levels by preventing the gluconeogenesis and/or glycogenolysis, thus avoiding hyperglycaemia, insulinresistance and hyperactivity of pancreatic β cells. In this study, insulinoresistance observed in dexamethasone treatment is associated with the imbalance in lipid metabolism characterized by the increase in total cholesterol, triglycerides, LDL cholesterol, atherogenic index and a decrease in HDL cholesterol. The plant extract at all doses prevented the increase in total cholesterol, LDL cholesterol and atherogenic index as in the case of *Garcinia* [16], *Cymbopogon citratus* (lemongrass oil) [17] and Naravelia zeylanica [18] treated rats . This result suggests that the plant extract may contain some secondary metabolites such as saponins, flavonoids, phenols and triterpenoids which are known to have hypolipidemic properties [19]. In this study we observed a significant increase in ALT and AST activities in dexamethasone+Dw treated group. This indicates hepatotoxicity which is related to glucotoxicity induced production of reactive oxygen species damaging cell functions and causing the leakage of these enzymes into the blood stream [8]. *H. noldea* aqueous extract improved ALT activities probably due to the improvement of blood glucose levels toward the normal value.The production of

reactive oxygen species (ROS) is confirmed in this study by the increase in MDA levels and the decrease in the SOD and catalase activities. It is well known that hyperglycaemia induced ROS generation which provoked lipoperoxidation, increasing antioxidant enzyme activities [20]. These antioxidant enzymes could also decrease due to their inactivation by ROS [21,22]. So the reduction of MDA levels and the prevention of the decrease of SOD and catalase activities by the plant extract; suggest the ability of *H. noldea* aqueous extract to counteract deleterious effects of dexamethasone induced hyperglycaemia associated to ROS generation.

Conclusion

The administration of dexamethasone increases blood glucose levels, insulin resistance, lipidemia, pancreatic islets area and depletion in glycogen. Concomitant treatment with dexamethasone and the aqueous extract of *Hibiscus noldea* prevented hyperglycaemia, hyperlipidemia, restored pancreatic islets area and improved hepatic glycogen content.

Acknowledgements

Authors are grateful for technical assistance received from the "Association pathologie cytologie developpement", Novartis, Bâle.This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Abbreviations

ALT: Alanine amino-transferase, AST: Aspartate amino-transferanse, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoproteins cholesterol, ROS: reactive oxygen species

Author's contributions

NTF, and DTJP carried out the study; FTN and DJH wrote the manuscript. DDPD carried out Histomorphometry of pancreatic islets, GKR and NTF helped to analyse data, KP and DT supervised the work. All authors read and approved the final manuscript.

References

[1]. Protzek AO, Rezende LF, Costa-Júnior JM, Ferreira SM, Capelli AP, Moura de Paula FM. Cristina de Souza J, Kurauti MA, Carneiro EM, Rafacho A, Boschero AC. Hyperinsulinemia caused by dexamethasone treatment is associated with reduced insulin clearence and lower hepatic activity of insullin-degrading enzyme. J Steroid. Biochem. 2016; 155: 1 – 8.

[2]. Nicod N, Giusti V, Besse C, Tappy L. Metabolic adaptations to dexamethasone-induced insulin resistance in healthy volunteers. Obes Res. 2003; 11 : 625–31.

[3]. Kooiji FO, Kal JE, Hans PC, Bonhomme VL. Blood glucose concentration profile after 10 mg dexamethasone in non-diabetic and type 2 diabetic. British J. of anaesth. 2006 ; 97 (6): 896-903.

[4]. Rafacho A, Cestari TM, Taboga SR, Boshero AC, Bosqueiro JR. High doses of dexamethasone induce increased β cell proliferation in pancreatic rat islets. Am J Physiol Endocrinol Metab. 2009; 296: E681 – E689.

[5]. Adjanohoun JE, Aboukakar N, Dramane K, Ebot ME, Ekpere, JA, Enow-Orock EG, Focho D, Gbile ZO, Kamanyi A, Kamsu KJ, Keita A, Mbenkum T, Mbi CN, Mbiele AL, Mbome IL, Mubiru NK, Nancy WL, Nkongmeneck B, Satabu B, Sofowora A, Tamze V, Wirmum CK. Traditional medicine and pharmacopoeia. Contribution to Ethnobotanical and Floristic Studies in Cameroon. Centre de Production de Manuels Scolaires, Porto-Novo (Rep. Du Benin), 1996 ; 641.

[6]. Chifundera K. Livestock diseases and the traditional medicine in the bushi area, kivu province, democratic republic of Congo. African Study Monographs. 1998; 19: 13 – 33.

[7]. Donfack JH, Nkenfou C, Boampong NG, Ngueguim TF, Ngadjui TB, Isha G, Van Reddy G, Inder PS, Rakesh S,. Ethnopharmacological investigation and In vitro antigardial activity of some Cameroonian medicinal plants. Pharmacologia. 2012; 3(12): 672-678.

[8]. Ngueguim TF, Esse CE, Dzeufiet DPD, Gounoue KR, Bilanda D C, Kamtchouing P, Dimo T. Oxidised palm oil and sucrose induced hyperglycemia in normal rats: effects of *Sclerocaryabirrea* stem barks aqueous extract. BMC Compl Alter Med. 2016 ;16:47. 1-11.

[9]. Youmbissi TJ, Djoumessi S, Nouedoui C, Ndobo P, Meli J. Profil lipidique d'un groupe d'hypertendus camerounais noirs africains. Médecine d'Afrique Noire. 2001; 48 : 305–314. *In French*

[10]. Odredra BR, Bates PC, Millward DJ. Time-course of the effect of catabolic doses of corticosterone on protein turnover in rat skeletal muscle. Biochem J. 1983; 214: 617 – 627.

[11]. Glass DJ. Signaling pathways that mediate skeletal muscle hypertrophy and atrophy. Nature Cell Biol. 2003; 5: 87 – 90.

[12]. Salman ZK, Refaat R, Selima E, El Sarha A, Ismail MA. The combined effect of metformin and L-cysteine on in flammation, oxidative stress and insulin resistance in streptozotocin-induced type 2 diabetes in rats. Eur J Pharmacol. 2013; 714(1–3): 448-455 .

[13]. Saad MJ, Folli K, Kahn JA, Kahn CR. Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats. J Clin Invest. 1993; 92: 2065 – 2072.

[14]. Turnbow MA, Keller SR, Rice KM, Garner CW. Dexamethasone down-regulation of insulin receptor substrat-1 in 3T3-L1 adipocytes. J Biol Chem. 1994; 269: 2516 – 2520.

[15]. Ruzzin J, Wagman AS, Jensen J. Glucocorticoid-induced insulin resistance in skeletal muscles: defects in insulin signalling and the effects of selective glycogen synthase kinase-3 inhibitor. Diabetologia. 2005; 48: 2119 – 2130.

[16]. Mahendran P, Devi, CSS. Effect of *Gracinia cambogia* extract on lipids and lipoprotein composition in dexamethasone administered rats. Ind J Physiol Pharmacol. 2001 ; 45: 345-350.

[17]. Kumar VR, Inamdar MN, Nayeemunnis, Viswanatha GL. Protective effect of lemongrass oil against dexamethasone induced hyperlipidemia in rats: possible role of decreased lecithin cholesterol acetyl transferase activity. Asian Pac J Trop Med. 2011. 658-660.

[18]. Rajakalanithi A, Swasthika P, Sujatha S. Evaluation of anti-hyperglycemic and anti-hyperlipidemic effects of Naravelia Zeylanica in streptozotocin-induced diabetic rats. Int J of Phytomedecine. 2016; 8:482-490.

[19]. Rajasekaran S, Jaykar B, Anandan R, Aboobacker S K, Vannamalar S.. Anti diabetic activity of leaves of *Zizyphus nummularia* by dexamethasone induced diabetic rat model. Inter J Phytopharmacol. 2013; 5(2): 844-851.

[20]. Gandhi RG, Ignacimuthu S, Paulraj MG. Hypoglycemic and b-cells regenerative effects of Aeglemarmelos (L.) Corr. bark extract in streptozotocin-induced diabetic rats. Food Chem Toxicol. 2012; 50(5): 1667-1674.

[21]. Aylin SD, Sereften A, Cemal C, Meltem S, Erdem Y. Effects of in vivo antioxidant enzyme activities of myrtle oil in normoglycaemic and alloxan diabetic rabbits. J.Ethnopharmacol. 2007; 110:498–503.

[22]. Marian V, Dieter L, Jan M, Mark TD, Milan M, Joshua T. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39(1): 44-84.