WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 11, 855-866 Research Article ISSN 2278 – 4357

PREVENTION OF DIABETE COMPLICATIONS BY HYDRO-ETHANOLIC EXTRACT OF THE LEAVES OF *TRILEPISIUM MADAGASCARIENSE* LEEUWENBERG D.C. (MORACEAE)

Raoul AMPA[1,2]*, Cyr Jonas MORABANDZA[2*], Gabriel AHOMBO[1], Etienne NGUIMBI[1], Martin DIATEWA[2], Ange Atoine ABENA[2], Théophile DIMO[3], Jean Maurille OUAMBA[4]

[1]Laboratory of Molecular and Cellular Biology, Faculty of Sciences and Technic, University Marien Ngouabi, P.O. Box 69, Brazzaville-Congo.

[2]Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, University Marien Ngouabi, P. O. Box 69, Brazzaville-Congo.

[3]Departement of Biology and Animal Physiology, Faculty of Sciences, Université of Yahoundé 1, Cameroon

[4]Vegetable Chemical and Life Unity, Faculty of Sciences and Technic, University Marien Ngouabi, P. O. Box 69, Brazzaville-Congo.

Article Received on
16 Sept. 2017,

Revised on 06 October 2017,
Accepted on 26 October 2017

DOI: 10.20959/wjpps201711-10363

***Corresponding Author**
Raoul AMPA
Laboratory of Molecular and Cellular Biology, Faculty of Sciences and Technic, University Marien Ngouabi, P.O. Box 69, Brazzaville-Congo.

ABSTRACT

This study was initiated to contribute to the prevention of diabetes complications. The rats are made diabetic by injection of streptozotocin (60 mg/kg) then oraly treated with hydro-ethanolic extract of *T. madagascariense* (400 mg/kg) leaves during 3 weeks. It come out that, hydro-ethanolic extract of *T. madagascariense* leaves at the used dose brings back glycemia towards the normal from the second week ; restores hepatic and renal functions by a significant descrease of creatinin ($p<0.05$) and plasmatic transaminases rates ($p<0.01$) compared to the untreated diabetic rats. The extract significantly standardizes ($p<0.01$) lipidic parameters, atherogenicity index and avoids the weight loss. These preliminary results suggest that the leaves of *T. madagascariense* are endowed of antidiabetic potentiality and, open a prospect towards the development of a traditional drug improved against diabetes and its complications.

KEYWORDS: *T. madagascariense*, leaves, hydro-ethanolic extract, diabetes.

INTRODUCTION

Considered as most frequent metabolic affection in the world, diabetes is caused by many genetic and environmental factors acting in synergy.[1] These factors indeed, induce an insufficiency or a misuse of insulin, which causes a glucidic metabolism disorder leading to a chronic hyperglycemia and, affecting the lipids and proteins metabolism. This abnormal metabolism is in origin of diabetic complications such as renal insufficiency, hepatic lesions, cardiovascular affections and, many other diseases. If at the man, the complications appear approximately ten years after installation of diabetes, in the rat made diabetic by administration of streptozotocine, they appear from the second week only which follows the installation of diabetes.[2,3,4] In this study, we undertook to evaluate some biochemical parameters after three weeks of treatment of diabetic rats with hydro-ethanolic extract of *T. madagascariense* leaves in order to research the capacity of the extract to protect diabetes complications. Thus, the evaluation of lipidic parameters (triglycerides, total serum cholesterol, HDL-cholesterol, LDL-cholesterol and index of atherogenicity), rates of aspartate aminotransférase (ASAT), alanine aminotransférase (ALAT) and serum creatinin were carried out to evaluate extract capacity respectively to prevent diabetic rats against cardiovascular complications, hepatic and renal damage and, weight loss. The objective of this study being to evaluate the protective effect of hydro-ethanolic extract of the leaves of *T. madagascariense* on diabetes complications in the Wistar rats.

MATERIEL AND METHODS

Plant material

T. madagascariense leaves were collected in September 2013 at Faculty of Science and Technic of Brazzaville (Congo). The sample was authenticated by National Research in Exacts and Naturals Sciences Institute (IRSEN) by comparison with the N° 3640 sample collected by J. Koechlin in Mars 1956 at Loudima (Department of Bouenza). The leaves were carefully washed and air dried, for two weeks; reduce in powder by using a wooden mortar. 100 g of powder was macerated for 72 h in 1000 ml of hydro-ethanolic solvent (800 ml ethanol /200 ml distilled water). The macerate was filtered (Whatman paper N°3), concentrated and dried at 55°C. 12 g of dry extract (12 % of extraction yield) were obtained and preserved for the various tests.

Experimental Animals

Albino male Wistar rats (*Rattus norvegicus*), from 2 to 3 months weighing between 250 and 300 g, raised with the animalery of the Laboratory of Animal Biology and Physiology of the University of Yaounde I were used for this work. Animals had free access to water and food.

Induction of diabetes of type 1

The diabetes of type 1 was induced by injection of 60 mg/kg of streptozotocine (SIGMA, Chemical-Co, the USA), beforehand dissolved in NaCl solution (0,9 %) in dorsal vein of penis of each animal after, anaesthesia with diethyl-ether. The tracking of diabetic rats was done 72 hours later. Were considered as diabetic and selected for experimental process, all animals which presented a higher glycemia after fasting or equalizes to 3 g/l. The control group received intravenous injection of NaCl solution (0,9 %) at the dose of 1 ml/kg after, induction of diabetes.

Evaluation of glycemia rate

Glycemia rate was performed by using a glucometer Accumulator-Check Activates (Rock diagnoses, France). A light incision of distal end of the tail by sterile blade made it possible to obtain with each measurement a drop of blood and this one being immediately deposited on the active beach of the strip of the Accumulator-check type.

Treatment of animals and blood analysis

4 groups of 5 male rats each one were treated for three weeks as follows: group 1 as normal rats treated with 10 ml/kg distilled water ; group 2 as diabetic rats treated with 10 ml/kg distilled water ; group 3 as diabetic rats treated with 5 UI/kg of insulin and group 4 as diabetic rats treated at 400 mg/kg of hydro-ethanolic extract of *T. madagascariense* leaves. Each morning, after weighing animals, water and extract were oraly administred and, insulin injected by subcutaneously. Glycemia of all animals was taken away at the end of each week, after 16 hours of fasting. At the 22^{nd} day of experience, the rats were anaesthetized using diethyl-ether and sacrificed by decapitation. The blood of each animal was collected in dry tubes, then centrifuged at 3000 rpm for 15 minutes. The collected serum were aliquoted and preserved at -20°C for biochemical parameters. After dissection of each rat, kidneys and liver were preserved and fixed in BOUIN liquid (at the portion of 2/3 liquid and 1/3 body) for the homogenates being used to evaluate biochemical parameters.

Biochemical markers of hepatic, renal functions and lipidic profile.

The serum and homogenates creatinin was performed by using Jaffé methods (Kit Jaffé Creatinin, Fortress Diagnostics, United Kingdom). The reagents of Fortress diagnoses Laboratory were used to quantified Aspartate AminoTransférase (ASAT), Alanine AminoTransférase (ALAT), triglycerids serum, total cholesterol serum and, HDL-Cholesterol. The LDL-Cholesterol rate and atherogenicity index were determined respectively by the following relations. [5]

LDL-Cholesterol (g/l) = Total Cholesterol – (HDL-Cholesterol +Triglycerids/22)

$$Index\ of\ atherogenicity = \frac{[Total\ cholesterol]}{[HDL]}$$

Body weight of animals was taken away at the end of each week before any treatment.

Statistical analysis

Statistical analysis was determined by using software SPSS ; the results reported as mean ± SEM. Analysis of variance (ANOVA) followed by Student-Fischer test were used to compare the results. ANOVA test was significant at least with the threshold $p<0.05$.

RESULTS

Effect on glycemia

Figure 1 presents the evolution of glycemia of diabetic rats (type 1) during the three weeks of treatment with differents products compared to the untreated diabetic rats. After the 2nd week of treatment, the glycemia continues to increase in untreated diabetic rats whereas it decreases significantly ($p<0.01$) in the treated diabetic rats with insulin (-28.39 %) and hydro-ethanolic extract (-17.36 %).

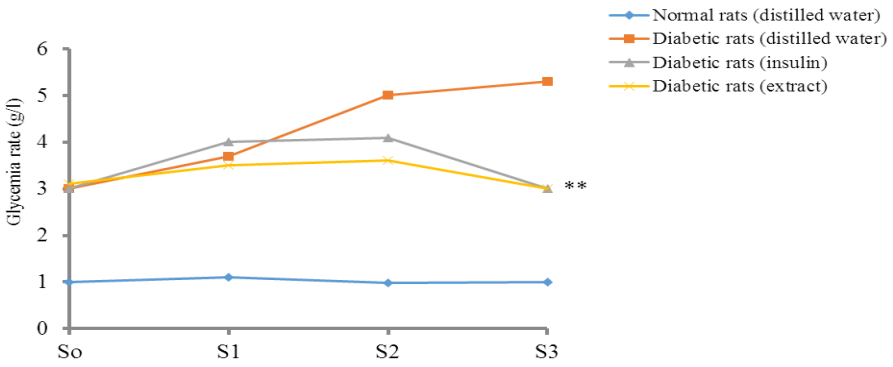

Figure 1: Effect of hydro-ethanolic extract of *T. madagascarience* leaves on glycemia of diabetic rats
Each point represents mean ± SEM, n=5; **p<0.01=significative difference compared to control diabetic at 3nd week.

Effect on creatinin rate

Figure 2 shows that creatinin rate is significantly higher ($p<0.01$) in untreated diabetic rats (0.450 ± 0.003mg/dl), compared to the treated diabetic rats with insulin (0.360 ± 0.020 mg/dl) ; hydro-ethanolic extract (0.360 ± 0.001 mg/dl) and that of normal rats received distilled water (0.250 ± 0.003 mg/dl).

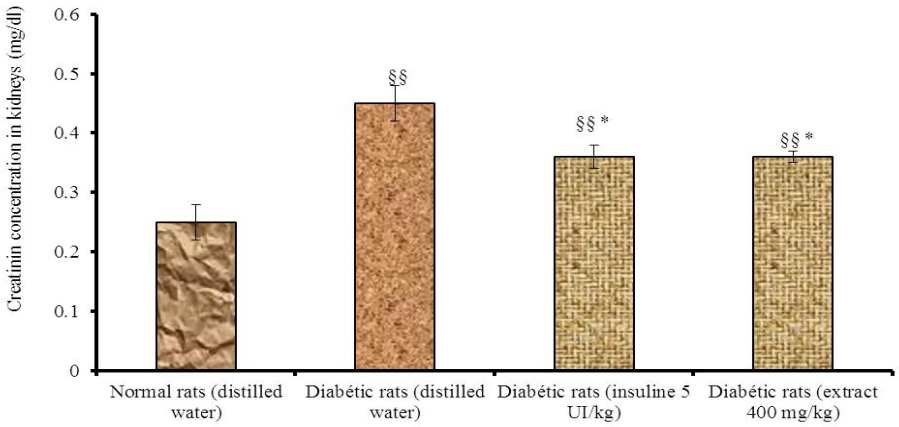

Figure 2: Effect of hydro-ethanolic extract of *T. madagascarience* leaves on creatinemy of kidneys' homogenates
Each bar, represents mean± SEM, n=5; §§*p<0.05=significative difference compared to diabetic contol

The results of Figure 3 revealed that creatinin of the treated rats with insulin (0.95 ± 0.07 mg/dl) and hydro-ethanolic extract (0.98 ± 0.01 mg/dl) are significantly low ($p<0.05$) compare to the diabetic rats having received distilled water (1.13 ± 0.07 mg/dl).

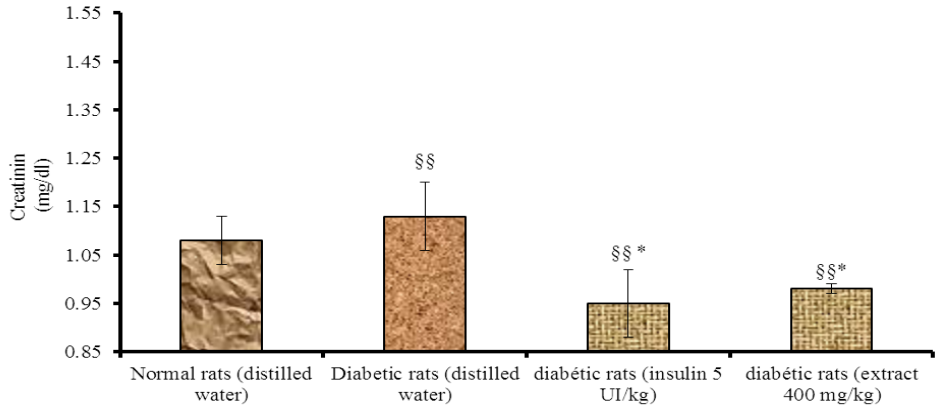

Figure 3: Effect of hydro-ethanolic extract of *T. madagascarience* leaves on creatinin of diabetic rats
Each bar represents mean ± ESM, n=5; §§*p<0.05=significative difference compared to diabetic rats and normal control

Effect on ALAT activity

Figure 4 shows that diabetic rats treated with insulin and hydro-ethanolic extract present a significant reduction ($p<0.01$) of ALAT activity, compared to the diabetic rats (distilled water).

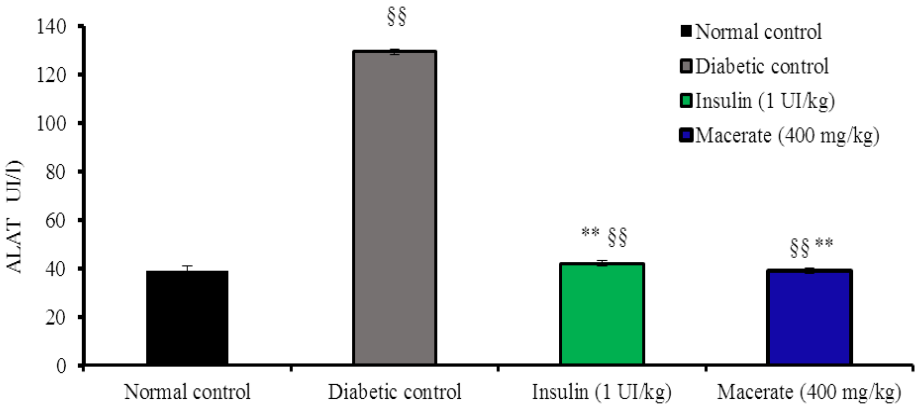

Figure 4: Effect of hydro-ethanolic extract of T. madagascarience leaves on ALAT activity of diabetic rats
Each bar represents mean± ESM, n=5; §§**p<0.01=significative compared to the control diabetic and normal control

Effect on serum ASAT activity

The results illustrated by the Figure 5 reveal that the treatment of diabetic rats by hydro-ethanolic extract or insulin is accompanied by a significant descrease of ASAT activity ($p<0.01$). ASAT activity of diabetic rats treated with hydro-ethanolic extract is significantly lower ($p<0.01$) than that of diabetic rats treated with insulin (52.77 ± 0.70 UI/l against 73.80 ± 0.61 UI/l).

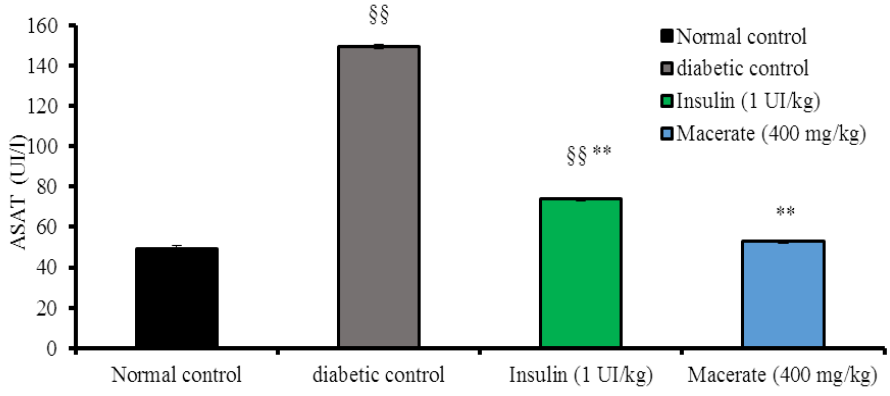

Figure 5: Effect of hydro-ethanolique extract of T. madagascarience leaves on ASAT activity of diabetic rats
Each bar represents mean ± ESM, n=5; §§**p<0.01=significative difference compared to control diabetic and normal control

Effect on lipidic parameters

Table 1 shows the results of treatment with extract (400 mg/ml) and insulin (1UI/kg). One observes a significant descrease ($p<0.01$) of triglycerid compared to the untreated diabetic rats. This descrease is more notable with the extract than that of insulin ($p<0.01$). Total cholesterol in diabetic rats receiving distilled water is significantly higher ($p<0.01$) than that of diabetic rats treated with insulin or extract which have rates closer to the normal rats. The treatment with insulin or extract induces a significant increase of HDL-Cholesterol rates in diabetic rats, compared to the diabetic rats with distilled water treatment ($p<0.01$). The LDL-Cholesterol rate is significantly higher ($p<0.01$) in diabetic rats receiving distilled water than in the diabetic rats treated with insulin or extract.

Table 1: Effect of hydro-ethanolic extract and insulin on lipidic parameters in diabetic rats.

Lipidic Parameters	Treatment of rats			
	Control (distilled water, 10 ml/kg)	Diabetic rats (distilled water, 10 ml/kg)	Diabetic rats insuline 5 UI/ kg/jour)	Diabetic rats (extract 400 mg/kg)
Triglycerids (g/l)	2.04±0.04	3.49±0.03 §§	2.26±0 ;30 **	1.93±0.33 **
Seric total Cholesterol (g/l)	0.52±0.01	0.77±0.01 §§	0.55±0.06 **	0.55±0.01 **
HDL-Cholesterol (g/l)	0.35±0.01	0.20±0.01 §§	0.31±0.02 **	0.55±0.01 ** §§
LDL-Cholesterol	0.14±0.01	0.40±0.01 §§	0.13±0.01 **	0.25±0.01 ** §§

n = 5; **$p<0.01$ = significant difference by comparison with diabetic rats having received distilled water ($p<0.01$); §§ = significant difference by comparison with the normal rats having received distilled water ($p<0.01$); HDL = high density lipoprotein; LDL = low density lipoprotein.

Effect on index of atherogenicity

The index of atherogenicity (figure 6) of diabetic rats treated with distilled water (3.77 ± 0.24) is significantly higher ($p<0.01$) than that of normal rats having received distilled water (1.53 ± 0.40) ; diabetic rats treated with insulin (1.73 ± 0.01) and hydro-ethanolic extract (0.98 ± 0.01).

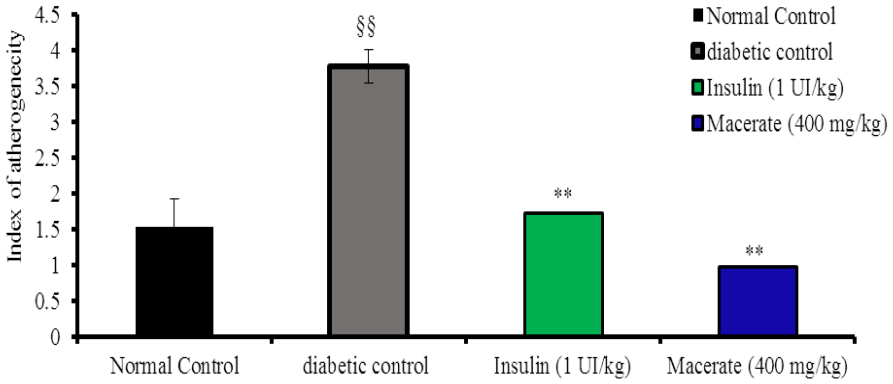

Figure 6: Effect of hydro-ethanolic extract of *T. madagascarience* leaves on the index of atherogenicity
Each bar represent mean ± ESM; n=5; **p<0.01= significative difference compared to control diabetic and normal control

Effect on body weight

Figure7 shows that the untreated diabetic rats present a descrease body weight of 27.12 %. Diabetic rats treated with 400 mg/ml of extract presents a tendency of stabilization of body weight compared to the initial weight. Whereas diabetic rats treated with insulin presents light increase of body weight (4,17 %) and normal rats have an increase of 17 %.

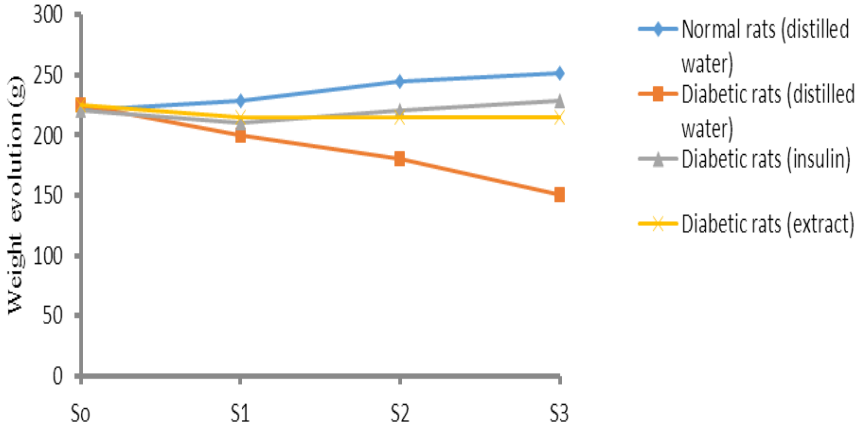

Figure 7: Effect of hydro-ethanolic extrac of *T. madagascarience* on weight evolution
Each point represents mean ± SEM, n=5; significative difference compared to diabetic rats

DISCUSSION

The object of this investigation was to research the protective effect of hydro-ethanolic extract of *T. madagascariense* leaves on installation of diabetes complications in the male rat. From results, it come out that hydro-ethanolic extract (400 mg/ml) activity in diabetic rats is observed after the 3rd week of treatment. The descrease of the average glycemia starts from the 2nd week of treatment (Figure1). Compared to that of methylen/methanol chloride of

Ceiba pentandra whose glycemia rate was standardized at 5th week for type1 and at the 3rd week for type 2 of treatment at the same dose[6] suggest that the extract of *T. madagascarience* would present an interesting effect. This reduction having lead to its standardization, would be probably due to the regeneration of β cells, which is allotted to the flavonoïdes.[7, 6] The regeneration would lead to the secretion of insulin necessary to control glycemia.[8] This phenomenon of regeneration of β cells of Langerhans was showed with the extracts of *Woodland Gymnema*, in the rats made diabetic with the streptozotocine. [9,10]

The reduction of creatinin rate observed in diabetic rats treated with the extract (400 mg/ml), testify to a restoration of renal function. The regeneration of β cells of Langherans by bioactives compounds present in the extract, which leads to the secretion of insulin, would be responsible of the restoration of renal function. Probably, secreted insulin would inhibit the catabolism of renal proteins and would thus have a positive effect on the regression of renal lesions induced by diabetes; that suggest a protective purpose of kidneys of diabetic rats by the extract. Similar results have been already reported by other studies. [11,12,13,14,15,16,17]

The complications of hepatic affections can result from diabetes.[18] The reduction of the transaminases activities observed in diabetic rats treated with extract (400 mg/ml), compared to the untreated diabetic rats, indicate that this extract may prevents hepatic lesions induced by diabetes. It thus improves the hepatic function, and it is not toxic on liver, but have a protective effect. Identical results were reported with the extracts of *Urena lobata* ,[13] *Achyranthes aspera*,[19] *Spondias mombin* and *Parinari polyandra* combined,[20] *Aristolochia indica*,[21] *Phyllanthus urinaria* and *Cassia auriculata*.[15,16] The phenomenon of regeration of β cells of Langherans would amongst other things explain hepato-protective effect of hydro-ethanolic extract in the treated diabetic rats.

Several studies reported that cardiovascular complications associated to diabetes are due to the disturbances of lipidic metabolism.[22,23] The significant increase ($p<0.01$) of HDL-cholesterol rate and the significant reductions ($p<0.01$) of triglycerids, total cholesterol, LDL-cholesterol rates and index of atherogenicity of diabetic rats treated with hydro-ethanolic extract of *T. madagascariense* at the dose of 400 mg/ml, compared to the untreated diabetic rats, indicate that the extract protects from the disturbance of lipidic metabolism induced by administration of streptozotocine. The same cardio-protector effect was observed with the extracts of *Caesalpinia bonduc*, [24] *Coriandrum sativum*, [25], *Senna auriculata*,[12] *Smallanthus sonchifolius*. [26] Once again, the regeneration of β cells of Langerhans would

explain the improvement of the rates of lipidic parameters observed in the treated diabetic rats. Indeed, the insulin secreted at the time of this phenomenon, would standardize the lipid level in treated diabetic rats.[10] Secreted insulin induced a lowering of the activity of enzymes implied in the biosynthesis of cholesterol ; affects the lipolysis and inhibits the secretion of VLDL, thus leading to non synthesis of LDL and the reduction of triglycerids and cholesterol rates. A probable regeneration of β cells by hydro-ethanolic extract provoke the restoration of renal and hepatic functions, justifying thus the stabilization of body weight of diabetic rats treated with extract.

CONCLUSION

The results obtained in the present work show that hydro-ethanolic extract of the leaves of *T. madagascariense* at the dose of 400 mg/kg prevents the diabetic rat of renal, hepatic and cardiovascular affections which inevitably joins the complication of diabetes.

REFERENCES

1. Balkau B., Charles M. A., Eschwège E. 2000. Discussion épidémiologique des nouveaux critères du diabète. Mt Endocrinologie, 2: 229-234.
2. Sokeng D. S. 1998. Essai thérapeutique des complications testiculaires du diabète chez le rat par les extraits d*'Anacardium occidentale* (Anacardiacées), plante médicinale. Thèse de 3ème cycle, Université de Yaoundé I, 150 p.
3. Ngueguim T. F. 2008. Effets hypoglycémiants des extraits de *Dorstenia picta* (Moraceae) et impact sur les complications du diabète de type 1. Thèse de Doctorat/ph D, Université de Yaoundé I, 202 p.
4. Jyoti M., Vihas T.V., Ravikumar A., Sarita G. 2002. Glucose lowering effect of aqueous extract of *Enicostemma littorale* Blume in diabetes: a possible mechanism of action. Journal Ethnopharmacology, 81: 317-320.
5. Chander R., Singh K., Khanna A. K., Kaul S. M., Puri A., Saxena R., BhatiaG., Rizvi F., Rastogi A. K. 2004**.** Antidyslipidemic and antioxidant activities of different fractions of *Terminalia arjuna* stem bark. Indian J Clin Biochem, 19(2): 141-148.
6. Dzeufiet- Djomeni P. D., Tedong L., Asongalem E.A., Dimo T., Sokeng S.D., Kamtchouing P. 2006. Hypoglycemic effect of methylene chloride / methanol root extract of *Ceiba pentandra* in normal and diabetic rats. Indian Journal of Pharmacology. 35: 194–197.

7. Marles R. J., Farnsworth N. R., 1995. Antidiabetic plants and their active constituents. Phytomedicin 2(2): 123 - 189.
8. Pandeya B.K., Tripathi P.I., Mishra K.M., Dwivedi N., Pardhi Y., Kamal A., Gupta P., Dwivedi N., Mishra C. 2013**.** A critical review on traditional herbal drugs: anemerging alternative drug for diabetes. International Journal of Organic chemistry, 3: 1-22.
9. Ali Ahmed A.B., Rao A.S., Rao M.V. 2010. *In vitro* callus and *in vivo* leaf extract of *Gymnema sylvestre* stimulate β-cells regeneration and antidiabetic activity in Wistar rats. Phytomedicine, 17(13): 1033-1039.
10. Vijayanand R., Aralelimath, Satish B. B. 2012. Antidiabetic effects of *Gymnema Sylvester* extract on streptozotocin induced diabetic rats and possible β-cell protective and regenerative evaluations. Digest Journal of Nanomaterials and Biotructures, 1(7): 135-142.
11. Mahera N.A. 2010. Histological and histochemical changes in diabetic male rat liver and intestine: and protective effect of *Cinnamon* oil. Journal Faculty of Medicine Baghdad, 52 (3): 366-371.
12. Shanmugasundaram R., Kalpana Devi V., Tresina Soris P., Maruthupandian A., Mohan V.R. 2011. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Senna auriculata* (L.) Roxb. Leaves in alloxan induced diabetic rats. International Journal of Pharm Tech Research, 2(3): 747-756.
13. Akhere A., Omonkhua, Onoagbe O.L. 2011. Evalauation of the long-term effects of *Urena lobata* root extracts on blood glucose and hepatic function of normal rabbits. Journal of Toxicology and Environmental Health Sciences 8(3): 204-213.
14. Fawzi I., Kamal M., Ahmad B.K., Talal A. 2012. Hepatoprotective, cardioprotective and nephroprotective actions of essential oil extract of *Artemisia sieberi* in alloxan-induced diabetic rats. International Conference on chemical, Environmental and Biological Sciences (ICCEBS, 2011), 362-366.
15. Munish G., Chanchal G. 2012. Effect of *Phyllanthus urinaria* in biochemical profile of experimental hyperglycemic albinos rats. Research Journal of Pharmaceutical Sciences, 1(1): 2-6.
16. Daisy P., Kani J.G.F. 2013. Hypolipidemic and hepatoprotective effects of *Cassia auriculata* Linn Bark extracts on streptozotocin induced diabetics in male Wistar albino rats. Asian Journal of Pharmaceutical and Clinical Research, 6(2): 43-48.
17. Muzammil Syed M., Dinesh V., Rahiman Maboop S., Rajesh S., Raj Paul J., Karthik V., Ashwaq Aadhil B., Kumar Prakash P., Kumaran S. 2013. Antidiabetic potential of herbal

extracts studied in (STZ) streptozotocin induced diabetic rat. International Journal of Pharmacy and Biological Sciences, 2(3): 78-85.

18. Levinthal G.N. and Tavill A.S. 1999. Liver disease and diabetes mellitus. Clinical Diabetes, 17(2): 1-20.
19. Mandar Z., Uma A.B., Somani R.S., Yegnanarayan R., Karuna A.T. 2011. Effects of treatment with *Achyranthes aspera* (Agadha) ethanol extract on various hematological and biochemical parameters in alloxan induced diabetic rats. IJPFR, 1(1): 42-52.
20. Emeka E.J.I., Funmilayo D.O. 2011. Hypoglycemic effect, biochemical and histological changes of *Spondias mombin* Linn. And *Parinari polyandra* Benth. Seeds ethanolic extracts in alloxan-induced diabetic rats. Journal of Pharmacology and Toxicology, 6(2): 101-112.
21. Cynthia M.J., Rajeshkumar K.T. 2012. Effect of aqueous root extract of *Aristolochia indica* (Linn) on diabetes induced rats. Asian Journal of Plant Science and Research, 2(4): 464-467.
22. Akuyam S. A., Isah H. S., Ogala W. N. 2007. Evaluation of serum lipid profile of under-five Nigeria Children. Annals of African medicine, 6(3): 119-123.
23. Gupta R. K., Kesari A. N., Diwakar S., Tyagi A., Tandon V., Chandra R., Watal G. 2008. In vivo evaluation of antioxidant and anti-lipidimic potential of *Annona scanosa* aqueous extract in type 2 diabetic models. Journal of ethnopharmacology, 118: 21-25.
24. Kishalay J., Kausik C., Tushar K.B., Soumyajit M., Debasis D., Kasi M.A., Debidas G. 2010. Antihyperglycemic and antihyperlipidemic effects of hydromethanolic extract of seed of *Caesalpinia bonduc* in streptozotocin induced diabetic male albino rats. International Journal of Pharm. Tech. Research, 2(4): 2234-2242.
25. Aissaoui A., Zizi S., Israili Z.H., Lyoussi B. 2011. Hypoglycemic and hypolipidemic effects of *Coriandrum sativum* L. in meriones shawi rats. Journal of Ethnopharmacology, 137(1): 652-661.
26. Habib C., Honoré S.M., Genta S.B., Sanchez S.S. 2011. Hypolipidemic effect of *Smallanthus sonchifolius* (Yacon) roots on diabetic rats: biochemical approach, chemico-biological Interactions, 194(1): 31-39.