Comparative Antidiabetic Potential and Survival Function of *Harungana madagascariensis, Physalis peruviana, Solanum americanum* and *Tithonia diversifolia* Extracts on Alloxan-Induced Diabetes in Guinea-Pigs

J.N. Kadima[1*], F.M. Kasali[2], B. Bavhure[2], A.O. Mahano[2], F.M. Bwironde[2]

[1]*Department of Pharmacology, School of Medicine and Pharmacy, University of Rwanda, Rwanda*

[2]*Department of Pharmacy, Faculty of Medicine and Pharmacy, Official University of Bukavu, DR Congo*

[3]*Department of chemistry, Faculty of Sciences, Official University of Bukavu, DR Congo.*

www.ijppr.humanjournals.com

ABSTRACT

Background-The World Health Organization recommends promoting traditional alternative herbal therapies in poor countries because modern drugs are out of reach for many populations. Objective-This study aimed at assessing antidiabetic potentials and survival function in alloxan-induced diabetic guinea-pigs treated during 28 days with *Harungana madagascariensis* Lama ex. Poir, *Physalis peruviana* (L.), *Solanum americanum*, and *Tithonia diversifolia* (Hem) A. Cray leaf-extracts. Methods- Animals were divided in 3 groups: negative control untreated group, reference group treated with glibenclamide 0.25mg/kg BW and test groups treated with plant extracts 200mg/kg BW. Blood samples were collected and glucose levels assayed at Day1, Day3, Day5, Day7, Day14, Day21 and Day28. Mean percentages of change (MPCs) in glucose level from Day1 baseline were calculated. Kaplan-Meier survival test was applied to compare time-to-death occurrence during the 28 days of treatment. Results-All plants reduced glucose levels significantly, but comparative MPCs between groups from Day7 to Day21 were significantly different (p<0.05). Kaplan-Meier Survival functions showed that all 6/6 animals in control group died within 10 days (censored=0%) while 1/6 death occurred in reference group (censored=83.3%); 2/3 animals died in *Solanum* group (censored=33.3%); 1/3 animal died in *Tithonia group* (censored=66.7%); 0/3 or no death occurred during the time of observation in *Harungana* and *Physalis* groups (censored=100%). Conclusion-All four studied plants showed potential antidiabetic activity consistent with studies by others, but differed in their capacity to prevent death. This can be in part related to the efficacy and toxicity of each plant components. Extensive study is needed to fix appropriate medications regimens.

1. INTRODUCTION

Diabetes mellitus is described as a complex chronic illness characterized by increased blood glucose levels resulting from the defect in insulin production or insulin action and it remains the most prevalent metabolic disorder in the world [1]. Globally in 2013, it was estimated that almost 382 million people suffer from diabetes for a prevalence of 8.3% [2]. In 2012, an estimated 1.5 million deaths were directly caused by diabetes and without intervention, this number is likely to increase more than twofold by 2030 [3].

The claimed intervention certainly requires continuous medical care with multifactorial risk-reduction strategies beyond pharmacological treatment [4]. The current pharmacological strategies include the administration of insulin for Type I diabetes and oral antidiabetic drugs for Type II. Oral antidiabetics include mainly the use of the sulfonyl urea derivatives like glibenclamide as insulin-releasing agents; biguanide derivatives like metformin acting mainly by increasing glucose consumption and reducing hepatic glucose build-up; alpha-glucosidase enzyme inhibitors like acarbose to reduce sugar absorption and new incretin mimetics (e.g. Exenatide) that mimic several of the actions of incretin hormones originating in the gut, such as glucagon-like peptide (GLP)-1, and Dipeptidyl peptidase-IV inhibitors (DPPI-IV e.g. saxagliptin) that suppress the degradation of many peptides, including GLP-1 [4].

Those modern drugs are however out of reach for many populations living in resources-limited countries. Further to the recommendation by the World Health Organization (WHO) to promote traditional alternative herbal therapies, a thousand ethnomedicinal researches have been undertaken, and many documented a considerable inventory of plants used worldwide in alternative health systems to manage diabetic patients [5-9]. More than 800 plant species have been found important sources for the discovery and development of new types of antidiabetic molecules [10].

This study was designed to seek out the antidiabetic potential effect of plants commonly used by regional traditional healers to treat diabetes mellitus and their capacity to prevent death in experimental induced-diabetic animals.

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

197

2. METHODS

2.1. Plant extracts

The used plants were collected in surrounding areas of Bukavu city in DR Congo. Fresh leaves of each plant were collected, shed-dried for one week, then ground in a mortar and finally sieved. Aliquots of crude powders were subjected to water extraction by maceration as commonly recommended [5, 6]. About 250 g of powdered leaves were soaked in 1500 ml of distilled water for 48 hours in the dark at room temperature and filtered with a filter paper. Thereafter the filtrates were dry-evaporated in an oven at 45˚C and the residues were reconstituted to a known concentration as dry extract in saline (1mg/ml).

2.2. Alloxan-induced diabetic animals

Guinea pigs of both sexes aged 3 to 4 months old and weighing 350 to 450 g were used. The animals were kept in the animal boundary of the Faculty of Medicine and Pharmacy at the Official University of Bukavu (UOB), prepared and used according to the standards required for experiment on laboratory animals [11]. A minimum number of animals had to be used in this explorative study to minimize unnecessary losses. Experimental diabetes was induced by s.c. injection of alloxan monohydrate (120 mg/kg BW) extemporaneously dissolved in physiological saline (10mg/ml) according to studies by others [12]. One week after, blood glucose test was performed using Glucometer (One Touch Ultra®) and animals presenting with blood glucose above 150 mg/dL were considered diabetic and recruited.

2.3. Evaluation of antidiabetic activity

The diabetic animals were segregated into homogenous groups (3 animals) based on the levels of glycemia. They were distributed in two series: (1)-baseline hyperglycemia < 200 mg/dL and (2)-baseline hyperglycemia >200 mg/dL at Day1 of treatment. Randomly, *Solanum* and *Physalis* extracts were set to series-1, and *Harungana* and *Tithonia* extracts to series-2. Each series was constituted of Control group (CG), Reference group (RG), and Test groups (TG). CG animals received only saline solution (1mL/100g BW) each day until death. RG animals were treated daily with oral glibenclamide solution (2.5 mg/100g BW). TG animals were treated daily with the plant extracts in equivalent dose of 200 mg/kg BW. Blood samples were collected and

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

198

glucose concentrations were measured with Glucometer^R at Day1, Day3, Day5, Day7, Day14, Day21 and Day28 respectively for each survival animal. The number of deaths was recorded each day.

2.4. Data analysis

Mean percentage of change (MPC) in daily glucose level from Day1 baseline was calculated for each group. Kaplan-Meier survival analysis was applied to compare death event between treatments. The analysis was done with SPSS v17 for Windows. Statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. Antidiabetic activity

Figure-1 shows daily glycemias as measured in survival animals. The mean glucose level in normal animals before any treatment was 86.8±9.4 mg/dL (71-96; n=24 animals); the mean alloxan-induced hyperglycemia one week after alloxan injection or Day1 of treatment was 232.5 ± 43.1 mg/dL (191 to 290; n=24 animals). The changes (mg/dL glucose) from Day1 to Day28 were respectively (199 to 85), (193 to 82), (191 to 95) in series-1 for glibenclamide, *Solanum*, *Physalis*; and (267 to 63), (253 to 101), (290 to 108) in series-2 for glibenclamide, *Tithonia*, and *Harungana*.
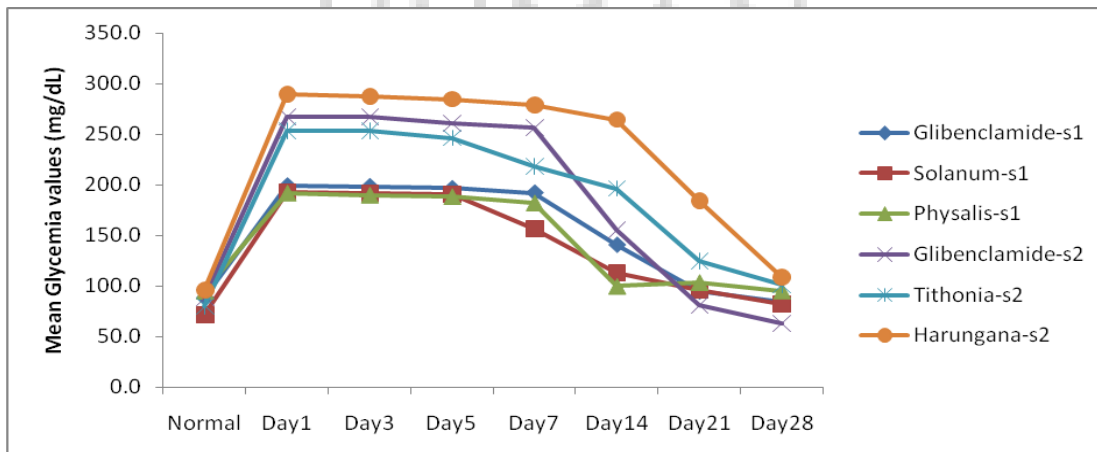

Figure 1. Mean glycemia values measured in survival diabetic guinea pigs treated orally with aqueous leaf extracts of the four mentioned plants and glibenclamide in series-1 and series-2

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

199

Figure-2 presents MPCs for each group. From Day1 to Day5, the difference in MPCs is not statistically significant. From Day7 to Day28, there were statistical significant differences between the four plants and glibenclamide ($p<0.05$). At Day7, *Solanum* and *Tithonia* MPCs were the highest (19% and 14%); at Day14, *Physalis* and *Solanum* MPCs were the highest (48% and 42%); at Day21, glibenclamide and *Tithonia* MPCs were the highest (62%; 51%); at Day28, glibenclamide and *Harungana* MPCs (64% and 62%) were the highest. Comparative MPCs at Day14 and Day28 were (8.7% and 62.4%) for *Harungana*, (22.7% and 60.2%) for *Tithonia*, (36.6% and 64.1%) for Glibenclamide, (41.6% and 57.6%) for *Solanum* and (47.8% and % 50.4) for *Physalis*. The estimated final MPCs at Day28 ranged from 50.4% to 64%, but in all survival animals the blood glucose concentration was almost returned to normal value.
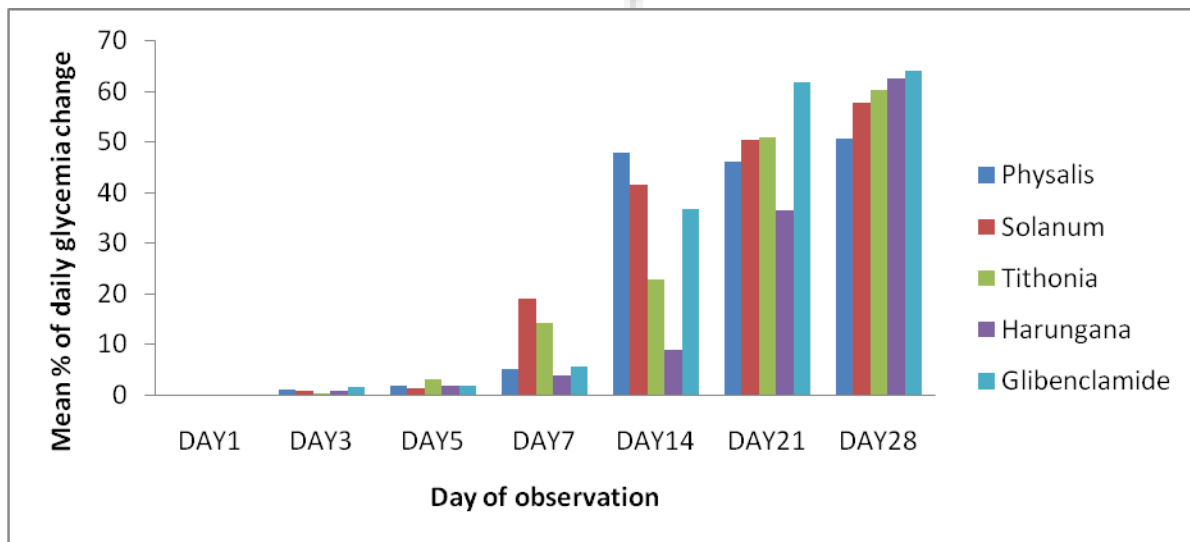

Figure 2. Mean percentage of change (MPC) in glycemia measured in censored animals from Day1 baseline to Day28. MPC= 100 x ((Glycemia at given day - Day1 baseline)/Day1 baseline)

3.2. Kaplan-Meier Survival Function

Table 1 shows Kaplan-Meier Cases processing summary. At Day28, 0/6 animals in control-untreated group survived (censored=0%); 5/6 in glibenclamide group survived (censored=83.3%); 1/3 in *Solanum* survived (censored=33.3%); 2/3 in *Tithonia* survived (censored=66.7%); 3/3 in *Harungana* and *Physalis* survived (censored=100%).

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

200

Table 1. Kaplan-Meier Survival function cases processing summary

Treatments	Total N	N of Events	Censored	N (%)
Control untreated	6	6	0	(0.0%)
Glibenclamide treated	6	1	5	(83.3%)
Solanum americanum treated	3	2	1	(33.3%)
Tithonia diversifolia treated	3	1	2	(66.7%)
Harungana madagascariensis treated	3	0	3	(100.0%)
Physalis peruviana treated	3	0	3	(100.0%)

As shown in Figure 3, all 6 control-untreated animals from series 1 and series 2 died within the first 10 days; one death in glibenclamide reference drug occurred after 21 days; two deaths in *Solanum* group occurred between 7 and 14 days; one death in *Tithonia* group occurred after 14 days. For *Harungana* and *Physalis,* there was no death during the 28 days time of observation.
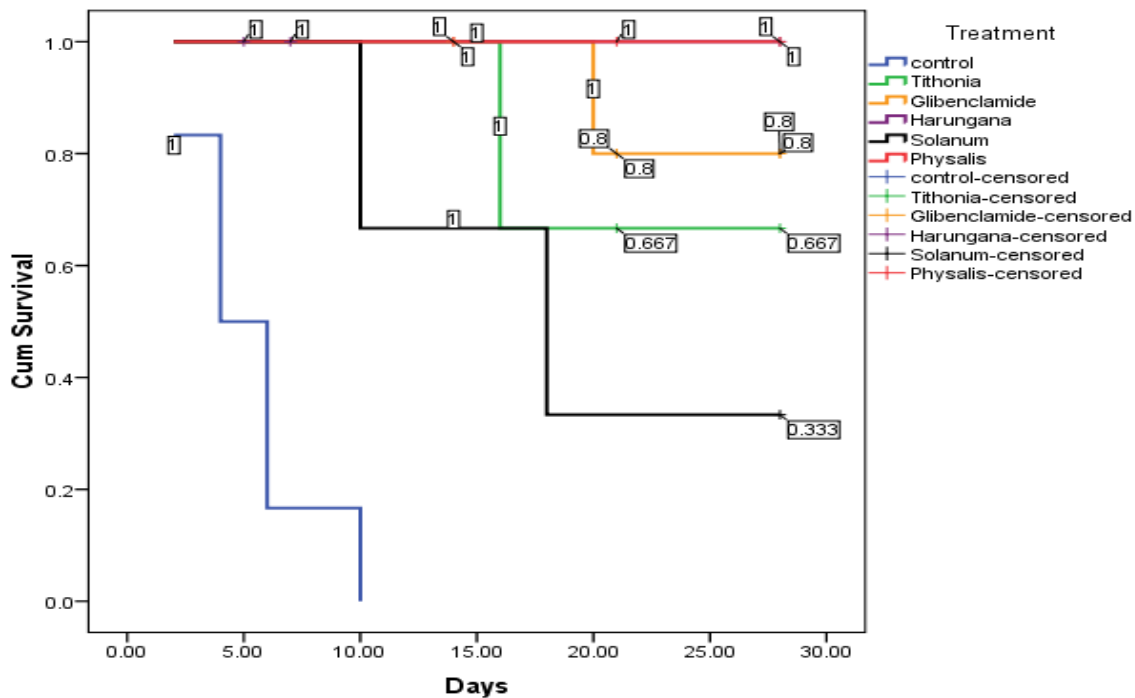

Figure 3 Kaplan-Meier Survival functions in alloxan-induced diabetic guinea-pigs treated with different plant extracts

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

201

4. DISCUSSION

Many studies have demonstrated hypoglycemic potential properties of the studied plants but no studies reported on animal survival. The findings in this study are consistent with studies by others regarding the percentage of reduction in glucose levels from baseline.

Studies on *P. peruviana* in rats and mice [13-16], humans [17] and *in vitro* molecular docking using Molegro Virtual Docke [18] demonstrated the antihyperglycemic activity of the fruits. Our previous study demonstrated the activity of leaf-extracts in single dose on oral glucose tolerance test (OGTT) [14], and in this study, repeated doses of leaf-extracts confirmed the previous result.

Studies of *H. madagascariensis* hydroethanolic extracts of stem bark showed their potential to reduce edema size formation and also the glucose levels in diabetic animals by inhibiting the activity of α-amylase [19, 20]. Those extracts decreased the food intake and body weight in the test group animals which was dose dependent, compared to the control group [21]. In our study, the aqueous leaf-extracts were used and also exhibited antidiabetic activity when administered daily at 200 mg/kg BW.

T. diversifolia has been commonly used in folk medicine to treat abscesses, microbiological infections, snake bites, malaria and diabetes. Anti-hyperglycemic studies of this plant showed significant activity in reducing blood levels of glucose, total cholesterol, triglyceride and low density lipoprotein-cholesterol (LDL-cholesterol) and increasing high density lipoprotein-cholesterol (HDL-cholesterol) in alloxan-induced diabetic mice and rats [22-24]. Phytochemical studies of the plant identified three sesquiterpenes [25] and two monoterpenes [26] that significantly increase glucose uptake in 3T3-L1 adipocytes without significant toxic effects *in vitro*. Most of the pharmacological activities have been attributed to sesquiterpene lactones (STLs) and some chlorogenic acid derivatives (CAs) in the leaves of this species. Our findings on antidiabetic potentiality of local plant species support those reports. Some studies on *Solanum genus* concluded that aqueous extracts of the leaves of *S. nigrum* are endowed with potential antihypertensive activity [27, 28]. *S. americanum* local species tested in the current study also is endowed with antidiabetic effect.

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

202

Concerning the survival functions, in spite of small number of animals used in this explorative study and the short period of observation, we found some significant differences in the survival profile (Figure 3). All untreated guinea-pigs died quickly in 10 days certainly due to hyperglycemia. However, it appeared from some above mentioned studies on alloxan-induced diabetic mice and rats that control untreated diabetic animals could survive beyond 21 days despite pancreas damage and high glucose levels (>350 mg/dL). This pushes to wonder whether guinea-pigs are more sensitive to hyperglycemia than mice or rats.

Administration of *Solanum* and *Tithonia* extracts resulted in early deaths compared to *Harungana* and *Physalis* extracts. Although deaths could be linked to various experimental factors or to sensitivity of individual animals used, we cannot rule out the contribution of the intrinsic efficacy and toxicity of each plant. *Harungana* and *Physalis* may be more efficient in regenerating pancreatic B cells activity and also less toxic at low doses. Toxicological studies have shown that the hydroethanolic extract of *H. madagascariensis* could be toxic only when consumed at high doses ($\geq$1.25 g/kg) [21]. The same observation was made for *P. peruviana* leaf-extracts [14].

Studies on the mechanisms of alloxan-induced diabetes showed that alloxan is not per se toxic [29]; rather it generates oxidant free radicals [30], triggers direct effect on islet cell permeability [31] and inhibits glucose-stimulated insulin release at the site of hexose transport [32]. Plant antioxidants such garlic, onion and fenugreek were found able to restore and regenerate pancreatic B cells by increasing the number of Langerhans islets [33]. Radical scavenging capacity was exhibited by increase in reduced glutathione (GSH) and a decrease in the concentrations of malondialdehyde (MDA) in alloxan-induced diabetic rats *in vivo* and against 1,1 Diphenyl-2-picrylhydrazy (DPPH) *in-vitro* [33]. It has also been postulated that the antidiabetic, antioxidant and anti-inflammatory activities of plant extracts could be complementary by reducing the high levels of inflammatory and oxidative stress markers which have been associated with the diabetic condition [34]. For instance, the main active constituents of *P. peruviana* are Physalins A, B, D, F and glycosides. It has been shown that Physalins B and F have a potent cancer suppressive activity by inhibiting the proliferation of lymphocytes, and also inhibit both the production of proinflammatory cytokines and activation of macrophages.

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

203

Physalins contained in *Physalis* can increase enzyme activity Superoxide dismutase (SOD) and catalase [35] to prevent free radicals damaging effect to pancreatic B cells.

This could possibly explain the ways the four studied plants also produce their effects against diabetic conditions. Those with less intrinsic toxicity and having high capacity of quick regeneration of Langerhans islets would have better survival profiles.

5. CONCLUSION

All four studied plants showed potential antidiabetic activity consistent with studies by others, but differed in their capacity to prevent death. This can be in part related to the efficacy and toxicity of each plant components.

ETHICAL ISSUES

Authorization was given by the Faculty Ethical Committee for animal experiment.

CONFLICT OF INTEREST

No conflict of interest.

6. REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030 *Diabetes Res Clin Pract* 2010; 87:4-14

2. Martiner. Prevalence of Diabetes in the World, 2013

http://healthintelligence.drupalgardens.com/content/prevalence-diabetes-world-2013

3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*, 2006, 3(11):e442.

4. American Diabetes Association. Standards of Medical Care in Diabetes. *Diabetes care* 2014, 37(1): S14-S80.

5. Kasali MF,Mahano AO, Bwironde FM, Amani AC, Mangambu JD, Nyakabwa D.S, Wimba LK, Tshibangu DST, Ngbolua KN, Kambale JK, Mpiana PT. Ethnopharmacological survey of plants used against diabetes in Bukavu city (D.R. Congo). *The Journal of Ethnobiology and Traditional Medicine. Photon* 2013, 119: 538-546.

6. Katemo M, Mpiana PT, Mbala BM, Mihigo SO, Ngbolua KN, Tshibangu DS, Koyange PR. Ethnopharmacological survey of plants used against diabetes in Kisangani city (DR Congo). *Journal of Ethnopharmacology* 2012; 144(1): 39-43.

7. Chan CH, Ngoh GC, Yusoff R. A brief review on antidiabetic plants: Global distribution, active ingredients, extraction techniques and acting mechanisms. *Pharmacogn Rev* 2012; 6(11): 22-28.

8. Firdous SM. Phytochemicals for treatment of diabetes. *EXCLI Journal* 2014; 13:451-453.

9. Yadav MK, Tripathi G and Tripathi P. Ethno medicinal plants with antidiabetic activity: a review *IJPSR, 2015; Vol. 6(11): 4570-4578*

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

204

10. Patel DK, Kumar R, Laloo D, Hemalatha S. Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects. *Asian Pacific Journal of Tropical Disease* 2012: 239-250.

11. EEC Directive. Guide for the care and use of laboratory animals.1986. [Online] Available from: http://ec.europa.eu/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf [Accessed on 13 May, 2013].

12. Rahimi P, Kabiri N, Asgary S, Setorki M. Antidiabetic effects of walnut oil on alloxan-induced diabetic rats. *African Journal of Pharmacy and Pharmacology* 2011; 5(2): 2655-2661.

13. Puente LA, Pinto-Muñoz CA, Castro ES, Cortes M. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. *Food Res Int* 2011*; 44*(7): 1733-1740.

14. Kasali MF, Kadima NJ, Mpiana PT, Ngbolua JPK, Tshibangu DS. Assessment of antidiabetic activity and acute toxicity of leaf extracts from *Physalis peruviana*(L.) in guinea-pig. *Asian Pac J Trop Biomed* 2013; 3(11): 885-890.

15. Hanaa FEM, Helmy HM, Abd El-Ghany MA. Antidiabetic and Antioxidative Activity of *Physalis* Powder or Extract with Chromium in Rats. *World Journal of Medical Sciences* 2012; 7 (1): 27-33.

16. Hassan AI, Ghoneim AMM. A Possible Inhibitory Effect of Physalis (*Physalis pubescens* L.) On Diabetes in Male Rats. *World Applied Sciences Journal* 2013; 21(5): 681-688.

17. Ulloa SLR, Ulloa ER. Effect of *Physalis peruviana* (Goldenberry) on postprandial glycemia in young adults. *Revista Médic aVallejiana* 2007; 4(1): 43-52.

18. Puspaningtyas AR. Docking studies of *Physalis peruviana* ethanol extract using molegro virtual docker on insulin tyrosine kinase receptor as antidiabetic agent. *International Current Pharmaceutical Journal* 2014; 3(5): 265-269.

19. Iwalewa EO, Adewale IO, Taiwo JB, Arogundade T, Osinowo A; Daniyan OM, Gbade E A. Effects of *Harungana madagascariensis* Stem Bark Extract on the Antioxidant Markers in Alloxan Induced Diabetic and Carrageenan Induced Inflammatory Disorders in Rats. *Journal of Complementary and Integrative Medicine* 2008; 5 (1): 1553-3840.

20. Momo CEN, Ngwa AF, Dongmo GIF, Oben JE. Antioxidant Properties and α-amylase Inhibition of *Terminalia superba, Albizia sp., Cola nitida, Cola odorata* and *Harungana madagascariensis* used in the Management of Diabetes in Cameroon. *Journal of Health Science* 2009, 55(5): 732-738.

21. Prosper Cabral N. Biapa, Julius E. Oben, Jeanne Y. Ngogang. Acute and Subacute Toxicy of Harungana madagascariensis Lama. *African Journal of Pharmaceutical Sciences and Pharmacy* 2012; 3: 45-57.

22. Montakarn T, Chunglok W, Kuanchuea R, Tangpong J. Antioxidant and Hypoglycemic Effects of *Tithonia diversifolia* Aqueous Leaves Extract in Alloxan-induced Diabetic Mice. *Advances in Environmental Biology* 2013, 7(9): 2116-2125.

23. Olukunle JO, Okediran BS, Sogebi EA, Eniope BJ. Hypoglycaemic and Hypolipidaemic Effects of the Aqueous Leaf Extracts of *Tithonia diversifolia. Annual Research & Review in Biology* 2014; 4(16): 2655-2662.

24. Zhao G, Li X, Chen W, Xi Z, Sun L. Three new sesquiterpenes from *Tithonia diversifolia* and their antihyperglycemic activity. *Fitoterapia* 2012; 83: 1590-7.

25. Xia L, Huang G, Guijun Zhao, Wansheng Chen, Junli Li and Lianna Sun (2013). Two New Monoterpenes from *Tithonia diversifolia* and Their Anti-Hyperglycemic Activity. *Rec. Nat. Prod*. 2013; 7(4) : 351-354.

26. Flávia DP, Rejane BO, Chagas-Paula DA, Gobbo-Neto L, Batista Da Costa F. Repeated-dose toxicological studies of *Tithonia diversifolia* (Hemsl.) A. gray and identification of the toxic compounds. *Journal of Ethnopharmacology* 2013; 147(2): 389–394.

27. Arumugam S.L, Manoharan KP, Rajinikanth R, Govarthanan M, Selvankumar T. Hypoglycemic and hypolipidemic activity of *solanum nigrum* in alloxan induced diabetic albino rats. *International Journal of Pharmaceutical Sciences and Research* 2012; 3(8): 2841-2848.

28. Laxmidhar M, Pattnaik S, Durga MK, Pratap KS, Sudam CS. Anti-Hyperglycemic potential of aqueous extract of leaves of *Solanum nigrum* Linn. *Der Pharmacia Lettre* 2010*; 2(3):168-179.

29. Wilson GL, Patton NJ, McCord JM, Mullins DW, Mossman BT (1984). Mechanisms of streptozotocin-andalloxan induced damage in rat B cells. Diabetologia, 27(6): 587-591

30. Houee C, Gardes M, Pucheault J, Ferradini C (1981).Radical chemistry of alloxan-dialuric acid: role of the superoxide radical, Bull. Eur. Physiopathol. Respir., 17: 43-48

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

205

31. Szudelski, T. The mechanism of Alloxan and Streptozotocin action in beta-cells of the rats' pancreas. *Physiol. Res.* 2011; 50: 536-546.

32. Rohilla A, Shahjad A. Alloxan Induced Diabetes: Mechanisms and Effects. *Int. J. Res. Pharm. Biomed. Sc.* 2012, 3(2): 819-823.

33. El–demerdash FM, Yousef M I, Abou El – Naga NI (2005). Biochemical study on the hypoglycemic effects of onion and garlic in alloxan –induced diabetic rats. Food Chem. Toxicol., 43: 57-63

34. Wu, S. J., Ng, L. T., Huang, Y. M., Lin, D. L., Wang, S. S., Huang, S. N., and Lin, C. C. (2005). Antioxidant activities of Physalis peruviana. Biol. Pharm. Bull.,28: 963-966.

35. Hui H, Tang G, Liang VWG. Hypoglycemic herbs and their action mechanisms. *Chinese Medicine* 2009, 4:1-11.

Citation: J.N. Kadima et al. Ijppr.Human, 2016; Vol. 5 (3): 196-206.

206