Phytochemical analysis and protective effect of Paliasa Leaves Extract (*Kleinhovia Hospita L.*) on pancreatic cytotoxicity in alloxan-induced hyperglycemic rats

Yuliana[1], Sianny Herawati[2]

[1]*Department of Anatomy, Faculty of Medicine, Universitas Udayana, Denpasar, Indonesia*

[2]*Department of Clinical Pathology, Faculty of Medicine, Universitas Udayana, Denpasar, Indonesia*

ABSTRACT

Diabetes mellitus is a worldwide problem nowadays. Many traditional plants are used to overcome the complication of this disease. One of them is paliasa leaves (Kleinhovia hospital L.). This study was planned to investigate phytochemical content and the protective effects of paliasa leaves aextract (*Kleinhovia hospita L.*) on pancreas cytotoxicity (hemorrhagic score and necrotic appearances) in alloxan-induced hyperglycemic rats. Male Wistar rats (three months old) were divided into four groups of seven animals each. Group I was diabetic control, Group II was diabetic groups, paliasa extract (300mg/kgBW) were given by sonde for a period of 14 days prior to alloxan injection (150 mg/kg intraperitoneal). Group III was diabetic rats given 600 mg/kgBW paliasa extract and group IV was diabetic rats given 900 mg for 14 days. At the end of the study, rats were sacrificed and pancreas was stained by hematoxylin eosin. Pancreas hemorrhagic score was divided into four groups, local (score 1), multifocal (score 2), extensive (score 3), and difuse (most severe, score 4). Microscopic examination was done using binocular *Olympus* microscope, enlargement 400 x. at Pathology Laboratorium Pegok, Denpasar, Bali. Data was analyzed by anova. Study showed that paliasa extract could lower hemorrhagic score on pancreas of diabetic rats, but not significant (p 0,205). Phytochemical analysis showed that paliasa leaves extract contained alkaloid, terpenoid, and flavonoid. Necrotic appearances were varied from pycnosis, karyorheksis, karyolysis, and vacuolization. In conclusion, paliasa leave extract has protective effect on pancreas cytotoxicity, however it was not significant.

Keywords: Alloxan, paliasa extract, diabetes, phytochemical, rats

ABSTRAK

Diabetes melitus merupakan masalah yang sering dijumpai di masyarakat Indonesia. Penyakit ini bisa menyebabkan komplikasi mikrovaskuler dan makrovaskuler. Pemberian obat tradisional seperti ekstrak daun paliasa (*Kleinhovia hospita L*.) diharapkan dapat menurunkan kadar glukosa darah dan mengurangi komplikasi. Tujuan
penelitian adalah mengetahui efektivitas pemberian ekstrak daun paliasa terhadap penurunan tingkat perdarahan pancreas pada tikus Wistar hiperglikemia yang diinduksi aloksan. Penelitian menggunakan rancangan *Control Group Design*, menggunakan 28 ekor tikus berusia tiga bulan. Tikus-tikus percobaan dibagi secara acak menjadi empat kelompok, yaitu kelompok kontrol, paliasa dosis 300mg/kg berat badan (BB), 600 mg/kg BB, dan 900 mg/kg BB. Setelah diberikan perlakuan selama 14 hari, tikus dieutanasia dan dilakukan pemeriksaan pancreas dengan pengecatan hematoxylin eosin. Tingkat perdarahan pancreas dibagi menjadi empat skor local (skor 1), multifocal (skor 2), ekstensif (skor 3), dan difus (paling berat, diberi skor 4). Pemeriksaan mikroskopis memakai mikroskop listrik binokuler *Olympus*, pembesaran 400 x. Pemeriksaan dilakukan di Laboratorium Patologi Balai Besar Veteriner (BB Vet), Pegok, Denpasar, Bali. Untuk mengurangi bias, pemeriksaan dilakukan dengan cara tersamar tunggal. Analisis data dilakukan dengan sidik ragam. Selain uji efek ekstrak paliasa terhadap perdarahan pancreas, pada penelitian ini juga dilakukan uji fitokimia kandungan ekstrak paliasa.
Hasil penelitian menunjukkan uji fitokimia ekstrak tanaman paliasa mengandung alkaloid, terpenoid, dan flavonoid. Ekstrak tanaman paliasa yang diberikan pada tikus hiperglikemi dapat menurunkan tingkat perdarahan, namun tidak bermakna (p 0,205).

Kata kunci: Aloksan, ekstrak paliasa, diabetes, fitokimia, tikus

Introduction

Diabetes mellitus is a worldwide disease and is depicted in blood glucose increasing above normal. World Health Organization predicted that more than 220 million people in this world suffer from diabetes and this number will be doubled in 2030 (Hamid and Moustafa, 2013). In Indonesia,

it is estimated that 50% of diabetic patients had not been diagnosed (Muhtadi *et al.*, 2015).

Oral anti diabetic and insulin sensitizer could repair insulin insensitivity, increase insulin production, and lower blood glucose. However, those medicine has some side effects such as hypoglycemia at higher dosage, low oral bioavailability, and low permeability when entering intestine epithelium. This will urge increasing demand of anti diabetic herbal product with fewer side effect (Malekpour *et al.*, 2012). One of traditional herbal plant that used to treat diabetes is paliasa (Dini, 2009).

Paliasa (*Kleinhovia hospital Linn*) is found at Sulawesi Selatan. (Ahmad, 2007). Paliasa leaves can be used as vegetables, supportive medicine for liver disease, and combat hair flea. In this study, we choose paliasa leaves because it is considered safer than medicine.

Diabetes mellitus induced by alloxan is type 1 diabetes mellitus, insulin dependent diabetes mellitus (Viana, 2004). It happened due to autoimun disorder and beta cell pancreas damage in Langerhans islets. First, beta cell pancreas decrease in amount and volume, then insulin deficiency will happen permanently (Waer and Helmy, 2012). Diabetes mellitus treatment without any side effects is a big challenge for future. WHO declared that anti-diabetic herbal medicine need special concerns (Patil *et al.*, 2011).

Hiperglycemia stimulates *reactive oxygen species* from many ways such as oxidative fosforilation, glucose autooxidation, lipooxygenase, and

nitrit oxide sintase. Natural anti oxidant in our body is not sufficient to attack injury due to free radicals. Thus, herbal anti oxidant is needed (Hamzah *et al*., 2012).

In diabetic rats, pancreas cells showed granular degeneration, pycnosis, karyorrhexis, and karyolysis (Liu *et al*., 2014). In histopathology of alloxan induced diabetic rats, we found changes in exocrine and pancreatic beta cells. On the islets of Langerhans cell degranulation occurs, accompanied by the formation of ghost islets with loss of whole cells. Bleeding is also found in all parts of the gland (Mir et al., 2013).

Material and methods

Plant Material Collection and Preparation

Paliasa leaves were harvested from Makasar. The process of extraction was as following: 1 kilogram of dry paliasa leaves, were poured with 15 litres methanol in room temperature for 14 hours, then they were filtered with Whatman paper. Filtrate was dried by vacuum through rotary evaporation (Arung *et al*., 2009).

Animals

Three months old male Wistar rats (180-200 g body weight) bred in our department animal house were used. The rats were housed in cages with room temperature. This experimental protocol was approved by Ethics

Committee of Animal Usage for Research and Education from Veterinary Faculty of Udayana University.

Chemicals

Alloxan was purchased from Pharmacological Laboratory of Widya Mandala University, Surabaya, Indonesia.

Experimental design

Those rats were randomly divided into four groups of seven each and induced with alloxan 120 mg/kgBW intraperitoneal in saline. All rats were found to be diabetic after 72 h. Rats with a blood glucose level ≥ 250 mg/ dL were considered to be diabetic.

Group I served as the diabetic control.

Group II were given paliasa leaves extract by sonde with dose 300 mg/kgBW,

Group III were given paliasa leaves extract by sonde with dose 600 mg/kgBW,

Group IV were given paliasa leaves extract by sonde with dose 900 mg/kgBW,

The curative effect of paliasa extract was then evaluated for a period of 14 days after alloxan administration and animals were chloroform. Pancreas were collected immediately for histopathological examination of hemorrhagic score.

Histopathological studies

Pancreatic tissues from all groups were subjected to histopathological studies. The whole pancreas from each animal was removed after sacrificing the animal under anesthesia, collected in 10% formalin solution. Sections of 5 μm thickness were cut and stained with hematoxylin and eosin (H and E) for histological examinations, i.e. pancreatic hemorrhagic score. Stained sections were qualitatively evaluated using an electric binocular microscope (Olympus).

Phytochemical analysis

Phytochemical analysis was done at Pharmacology Department Udayana University.

Atsiri oil

Extract is added with etanol. If it has aromatic smell, it will be steamed (evaporated) until dry. If the result still showed aromatic smell, then it was positive for atsiri oil.

Alkaloid

Two mL extract is steamed until it get residu. Residu is added with 5 mL HCl 2N, then it is divided to 5 reaction tube. First tube is added with weak acid that acted as blanko. Second tube is added with 3 drops Dragendorrff 3 drops, third tube is added 3 drops Mayer reaction, fourth tube is added wagner reaction. Orange sediment in second tube and white yellowish sediment in third tube means there is alkaloid.

Sterol and terpenoid

One ml extract is steamed, then it is added by 0.5 mL chloroform, and added by 0.5 mL anhidrat acetic acid. Then it is dropped by 12 mL sulphate acid. If the colour is green bluish, it showed sterol. If it showed brownish or violet ring in the boundary, there is terpenoid.

Saponin

Three mL extract is shaken vertically for 10 seconds, then we wait for 10 seconds. If there is stable foam for about 15 minutes, it means there is saponin. If we add 1 drop HCL 2 N, the foam is not dissapeared.

Polyphenol

One mL extract is added with iron/ferri (III) chloride 10%, if it turns to dark blue, blue black or black green, then it means there is polyphenol there.

Glycoside

It is done by Liebermann-Burchard reaction. One mL extract is evaporated, the rest is soluted in 5 mL anhidrate acetat acid, then added with 10 drop sulfate acid. It is considered positive if the colour turns to green or blue.

Flavonoid

One mL extract solution is evaporated, the rest is wetted by aseton, enhanced by a little of smooth borac acid powder and oxalic acid, then it is heated carefully. After that, the rest is mixed with 10 mL eter. By using

UV_{366}; if it is shown intensive yellowish fluorescence, then it means there is flavonoid.

Statistical analysis

All the grouped data were statistically evaluated with one way analysis of variance (ANOVA). $P < 0.05$ was considered statistical significant.

Results

Pancreas hemorrhagic score was divided into four groups, i.e. local (score 1), multifocal (score 2), extensive (score 3), and diffuse (score 4). Result of treatment effect was shown on table 1 (electric microscope, 400x)

Table 1

Mean Pancreatic Hemorrhagic Score after Treatment

Group	N	Mean pancreatic hemorrhagic score	SD	F	Study result
Control	7	3,14	0,690	1,647	0,205
Paliasa 300	7	2,71	0,756		
Paliasa 600	7	2,57	0,535		
Paliasa 900	7	2,43	0,535		

The table above shows that the mean pancreatic hemorrhagic score between groups after being given treatment did not differ significantly ($p > 0.05$).

Necrosis appearance were varies, from pynosis, karyolysis, and karyorhexis as shown in Figure 1. Phytochemical test showed that paliasa contains alkaloid, terpenoid, and flavonoid (Table 2).

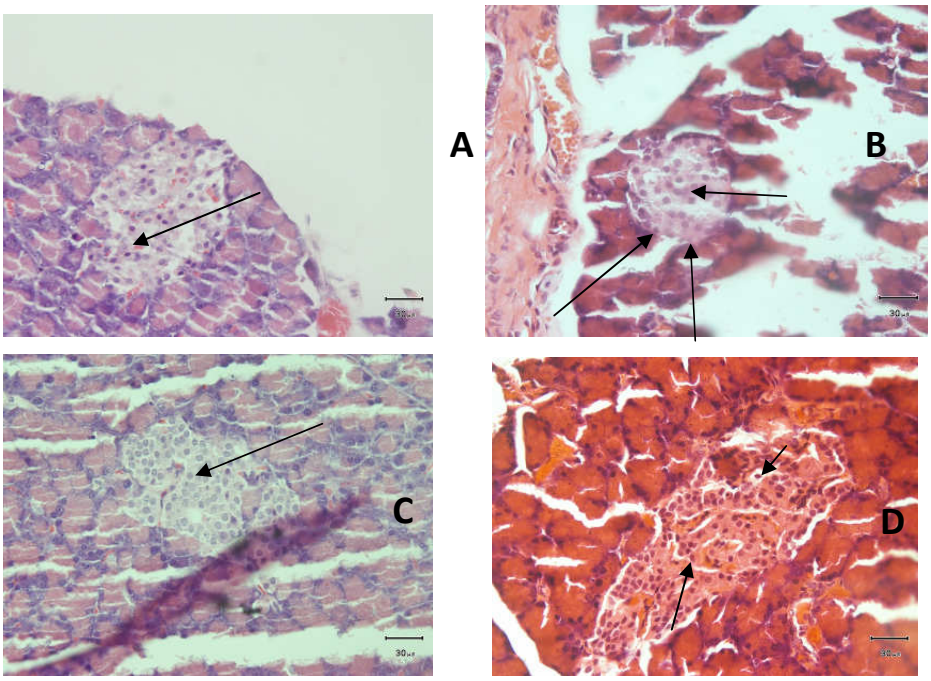

Figure 1. Necrosis appearance in pancreas of four study group (HE, 400x). Figure A is pancreas of hyperglycemic (arrow showed karyorhexis). Figure B is pancreas of hyperglycemic rats with 300mg/kgBW paliasa extract (there is pycnosis, karyorhexis, and karyolysis). Figure C is pancreas of hyperglycemic rats with 600mg/kgBW paliasa extract (there is karyorhexis). Figure D is pancreas of hyperglycemic rats with 900mg/kgBW paliasa extract (there is pycnosis and vacuola).

Table 2. Phytochemical test result

Number	Phytochemical content	Reference	Paliasa extract	
			Result	meaning
1	Atsiri oil	Aromatic smell	No aromatic smell	(-)
2	Alkaloid	Orange sediment (Dragendorff reaction)	No sediment	(-)
		Yellow sediment (Mayer reaction)	White yellowish sediment	(+)
		Brown sediment (Wagner)	Brown sediment	(+)
3	Sterol and Terpenoid	Blue green ring (Sterol)	-	(-)
		Brown or violet ring (Terpenoid)	Brown ring	(+)
4	Saponin	Foam 1-10 cm for 10 minutes and persist after adding 1 drop HCl 2N	No foam	(-)
5	Polyphenol	Dark blue or black greenish colour	No black greenish colour	(-)
6	Glycoside	Blue or green color	Black brown colour	(-)
7	Flavonoid	Intensive yellowish fluorescence at UV_{366}	Yellowish fluorescence	(+)

Discussion

Alloxan is beta cytotoxin. Alloxan chemically induced diabetes by destroying beta cells that produce insulin. A decrease in the release of insulin lowers insulin usage by the tissues. Increased oxygen free radicals occurs due to high blood glucose levels. Auto oxidation cause free radicals and this is exacerbated by the effects of diabetogenic alloxan (Hamzah et al., 2012).

Alloxan and its reduction product, dialuric acid, cause a redox cycle through the formation of superoxide radicals. Superoxide radicals experience dismutase become hydrogen peroxide. Highly reactive hydroxyl radicals are formed by Fenton reaction (Rohilla and Ali, 2012).

The effects of reactive oxygen species (ROS) along with an increase in cytosolic calcium concentration would lead to massive destruction of beta cells rapidly (Hamid and Moustafa, 2013). The severity of tissue damage and the degree of oxidative stress may depend on an imbalance between excessive ROS production and antioxidant defenses in the cells of the pancreas (Kikumoto et al., 2010). ROS are involved in various signaling pathways of angiotensin II. Increased activity of the renin-angiotensin system locally on the pancreas will increase pancreatic damage induced by ROS (Leung, 2007).

Beta cells of diabetic mice showed vacuolization and a decrease in secretory granules, fusion of some granules and pycnotic nuclei (Hamid and Moustafa, 2013). Giving alloxan causes morphological changes in diabetic rats in the form of severe damage to the beta cells of the pancreas, which is characterized by a decrease in the number of cells, cell damage, and even cell death (Dahecha et al., 2011).

Necrosis is usually found in the middle of Langerhans islet because this part is that initially influenced by alloxan (Singh and Gupta, 2007). Karyolysis, the loss of the cell nucleus, as well as the presence of remnants (residue) cells that have vacuole shaped found in research Jelodar et al. in 2005. At the core of beta cells that undergo kariolisis, also showed debris in the form of the fragment mass surrounding the cell nucleus (Boudreau et al., 2006).

Pancreas is more susceptible to oxidative stress compared with other organs as the cells of the pancreas have anti-oxidant enzymes lower (Robertson, 2006). Oxidative stress induced by alloxan will cause breakdown of DNA and activation polysintetase resulting in impaired synthesis of insulin in the pancreas (Takemoto et al., 2014). Pancreas function as a glucose sensor and insulin-requiring oxygen-rich environment as well as glucose, so that it can generate sufficient signal for insulin secretion and providing adequate insulin to target tissues. These special circumstances make pancreatic beta cells are highly susceptible to oxidative

stress (Lenzen, 2008). Hyperglycemic conditions lead to an increase in the concentration of H2O2 and induction of catalase activity as a defense mechanism against free radicals. However catalase levels may not be sufficient to prevent the onset of diabetes (Kikumoto et al., 2010).

Death of pancreatic beta cells in diabetes occurs due to contact with macrophages and T cells, and / or exposure to mediators such as cytokines, nitric oxide, and oxygen free radicals. Apoptosis was predicted as the main cause of death of pancreatic beta cells and the process was arranged by specific gene (Yukimoto, et al., 2010).

Vacuolization is one indication of structural disruption of membrane permeability, cause increase in fluid and electrolyte transport into the cell. Permeability disorders occur due to reactive oxygen species (Hamid and Moustafa, 2013). Variations of histopathological pancreas may occur because of differences in the status of anti-oxidants, as well as the size and maturity of cells (Mir et al., 2013).

Anti-oxidants contained in the plant act to neutralize free radicals. Free radicals associated with the development of degenerative diseases such as diabetes and cardiovascular disease (Renjith et al., 2013). Phytochemical test results paliasa plant contains alkaloids, terpenoids and flavonoids. Alkaloids may act to stimulate the multiplication of existing cells of Langerhans and differentiation into new cells. Alkaloids maybe also important for the recovery of partial beta cell (Singh and Gupta, 2007).

Alkaloid has anti hyperglycemic, anti-inflammatory, and anti-oxidants (Adeneye and Crook, 2015). Alkaloids lower insulin-mediated glucose disposal and reduce activities of two enzymes that are essential for the production of glucose, namely glycogen phosphorylase and glucose-6-phosphatase (Ezuruike and Prieto, 2014).

Terpenoids and flavonoids have anti-diabetic properties. Flavonoids are able to regenerate the damaged beta cells in alloxan-induced diabetic rats and act as insulin secretagogous (Balamurugan et al., 2014). Liu et al. (2007) found that flavonoids can enhance insulin sensitivity. Terpenoids and flavonoids may be able to improve the integrity of the B-endocrinocyte through increased release of insulin or insulin activity improvement (Snigur et al., 2008).

Flavonoids can modulate the activity of glycolytic and gluconeogenic enzymes (Renjith and Rajamohan, 2012). Flavonoids inhibit the glucose transporter epithelial cells of the small intestine and regenerate the damaged beta cells in the pancreatic beta cells induced alloxan (Sharma et al., 2013). The formation of advanced glycated end products (AGEs) can be prevented, and there is no atherosclerosis, nephropathy, and retinopathy (Rahimi, et al., 2005).

Efficacy hypoglycemic herbs are obtained through a process of increasing insulin secretion, inhibit glucose absorption from the intestine, inhibits glucose production from hepatocytes, and increase glucose uptake

by muscle and fat (Hui et al., 2009). Giving phytonutrients may be an effective strategy to overcome the complications of diabetes by regulating the enzyme responsible for metabolizing glucose (Renjith and Rajamohan, 2012). The active substance in traditional crops can activate the regeneration of pancreatic beta cells (Jelodar et al., 2005). Pancreatic function recovery occurs through the facilitation of metabolites in insulin-dependent process (Balamurugan, 2014).

Conclusion

The conclusion obtained from the study are:

1). Paliasa plant extracts (Kleinhovia hospital L.) contain alkaloids, terpenoids and flavonoids.

2). Paliasa plant extract given to the mice of hyperglycemia can reduce the level of bleeding pancreas, however it was not significant.

Acknowledgement

The authors are grateful to Udayana University for the financial assistance, with Contract Letter number 1438/UN14.2/PNL/01.03.00/2015 (Donation of Study Program).

REFERENCES

Adeneye, A.A., Crook, P.A. 2015. Weight losing, antihyperlipidemic and cardioprotective effects of the alkaloid fraction of *Hunteria umbellata* seed extract on normal and triton-induced hyperlipidemic rats. Asian Pacific Journal of Tropical Biomedicine 5(5): 387-94.

Arung, E.T., Kusuma, I.W., Purwatiningsih, S., Roh, S.S., Yang, C.H., Yang, C.H., Kim, Y.U., Sukaton, E., Susilo, J., Astuti Y., Wicaksono, B.D., Sandra, F., shimizu, K., Kondo, R. 2009.Antioxidant Activity and Cytotoxicity of the Traditional Indonesian Medicine Tangohai (Kleinhovia hospita L.) Extract. J Acupunct Meridian Stud (4): 306-8.

Balamurugan, K., Nishantini, A., Mohan, V.R. 2014. Antidiabetic and Antihyperlipidemic activity of ethanol extract *Melastoma malabathricum* Linn. Leaf in alloxan induced diabetes rats. Asian Pas J Trop Biomed 4 (Suppl 1): S442-8.

Boudreau, M.D., Taylor, H.W., Baker, D.G., Means, J.C. 2006. Dietary exposure to 2-aminoanthracene induces morphological and immunocytochemical changes in pancreatic tissue of fisher-344 rats. Toxicol. Sci. 93: 50-61.

Dahecha, I., Belghitha, K.S., Hamdenb, K., Fekib, A., Belghithc, H., Mejdoub, H. 2011. Oral administration of levan polysaccharide reduces

the alloxan-induced oxidative stress in rats. Int. J. Biol.Macromol (49):942–7.

Dini, I. 2009. Toksisitas Senyawa Metabolit Sekunder dari Ekstrak Kloroform Kulit Batang Tumbuhan Paliasa terhadap Artemi Salina Leach. Bionature Vol. 10(1):16-9.

Ezuruike, U.F., Prieto, J.M. 2014. The use of plant in the traditional management of diabetes in Nigeria: Pharmacoogical and toxicological considerations. Journal of Ethnopharmacology 155: 857-924.

Hamid, M.A., Moustafa, N. 2013. Protective effect of curcumin on histopathology and ultrastructure of pancreas in the alloxan treated rats for induction of diabetes. The Journal of Basic and Applied Zoology 66: 166-79.

Hamzah, R.U, Odetola, A.A, Erukainure, O.L., Oyagbemi, A.A. 2012. Journal of Acute Disease 135-140.

Hui, H., Tang, G., Go, V.L.W. 2009. Hypoglycemic herbs and their action mechanisms. Chinese Medicine 4:1-11.

Jelodar, G.A., Maleki, M., Motadayen, M.H., Sirus, S. 2005. Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. Indian J Med Sci 59: 64-9.

Kikumoto, Y. *et al.* 2010. Sensitization to alloxan-induced diabetes and pancreatic cell apoptosis in acatalasemic mice. Biochimica et Biophysica Acta 1802 (2010): 240-6.

Leung, P.S. 2007. The physiology of local renin-angiotensin system in the pancreas.J. Physiol. 580: 31-7.

Lenzen, S. 2008. Oxidative stress: the vulnerable beta-cell. Biochem. Soc. Trans 36: 343-7.

Liu, I.M., Tzeng, T.F., Liou, S.S., Lan, T.W. 2007. Myricetin, a naturally occurring flavonol, ameliorates insulin resistance induced by a high-fructose diet in rats. Life Sciences 81: 1479–88.

Liu, H.Y., Fang, M., Zhang, Y.Z. 2014. In Vivo Hypoglycaemic Effect and Inhibitory Mechanism of the Branch Bark Extract of the Mulberry on STZ-Induced Diabetic Mice. The Scientific World Journal 1-11.

Malekpour, A., Dehghani, S., Zahedi, S., Eskandari, F. 2012. Effects of Hydro-Ethanol Extract of *Myrtus communis* L. on Blood Glucose Level and Histopathological Changes in Alloxan-Induced Diabetic Rats. MEJSR 12(4): 517-22.

Mir, M.S., Darzi, M.M., Khan, H.M., Kamil, S.A., Sofi, H.A., Wani, S.A. 2013. Patomorphological effects of Alloxan induced acute hypoglycaemia in rabbits. Alexandria Journal of Medicine 4: 343-53.

Patil, R.N., Patil, R.Y., Ahirwar, B., Ahirwar, D. 2011. Evaluation of antidiabetic and related actions of some Indian medicinal plants in diabetic rats. Asian Pacific Journal of Tropical Medicine 2011: 20-23.

Rahimi, R., Nikfar, S., Larijani, B., Abdollahi, M. 2005. A review on the role of antioxidants in the management of diabetes and its complications. Biomedicine and Pharmacotherapy 59: 365–73.

Renjith, R.S., Rajamohan, T. 2012. Protective and curative effects of *Cocos nucifera* inflorescence on alloxan-induced pancreatic cytotoxicity in rats. Indian J Pharmacol 44:555-9.

Renjith, R.S., Chikku, A.M., Rajamohan, T. 2013. Cytoprotective, antihyperglycemic, and phytochemical properties of *Cocos nucifera* (L.) inflorescence. Asian Pacific Journal of Tropical Medicine 2013: 804-10.

Robertson, R.P. 2006. Oxidative stress and impaired insulin secretion in type 2 diabetes. Curr. Opin. Pharmacol. 6 (6): 615–619.

Sharma, N., Gupta, P.C., Rao, R.V. 2013. In-vitro antiradical and inhibitory potential of *Pentapetes phoenicea* Linn. Leaves against digestive enzymes related to diabetes. JOPR 569-72.

Singh, N., Gupta, M. 2007. Regeneration of β cells in islets of Langerhans of alloxan diabetic rats by acetone extract of *Momordica charantia* (Linn.) (bitter gourd) fruits. IJEB 45: 1055-62.

Snigur, G.L., Samokhina, M.P., Pisarev, Spasov, A.A., Bulanov. 2008. Structural alterations in pancreatic islets in streptozotocininduced diabetic rats treated with bioactive additive on the basis of Gymnema sylvetre. Morfologia 33(1): 60-64.

Takemoto, K., Doi, W., Zukeran, A., Inoue, J., Ishihara, K., Masuoka, N. 2014. Effect of *Aspergillus awamori*-Fermented Burdock Root on Mouse Diabeted Induced by Alloxan – Prevention of Cell Apoptosis. Food and Nutrition Sciences 5: 1554-60.

Viana, G.S., Medeiros, A.C., Lacerda, A.M., Leal, L.K., Vale, T.G., Matos, F.J. 2004. Hypoglycemic and anti-lipemic effects of the aqueous extract from Cissus sicyoides. BMC Pharmacol 8: 4-9.

Waer, H.F., Helmy, S.A. 2012. Cytological and Histochemical Studies in Rat Liver and Pancreas during Progression of Streptozotocin Induced Diabetes and Posiible Protection of Certain Natural Antioxidants. J Nutr Food Sci 2(9): 165-172.