Original article

# The protective effect of ethanol root extract of *Sphenocentrum jollyanum* on the morphology of pancreatic beta cells of alloxan challenged rabbits

**Mbaka, GO.[1]*, Adeyemi, OO.[2], Ogbonnia, SO.[3], Noronha, CC.[4] and Okanlawon, OA.[4]**

[1]Departament of Anatomy, Olabisi Onabanjo University, Remo Campus, Ogun State, Nigeria
[2]Departament of Pharmacology, College of Medicine, University of Lagos, Lagos, Nigeria
[3]Departament of Pharmacognosy, University of Lagos, Lagos, Nigeria
[4]Departament of Anatomy, College of Medicine, University of Lagos, Lagos, Nigeria
*E-mail: mbaaka@yahoo.com

## Abstract

Objective: To assess the protective role of *Sphenocentrum jollyanum* (SJ) root on beta cells against alloxan challenged diabetic rabbits. Methods: Rabbits of both sexes randomly divided into 6 groups (8 per group), received oral treatment as follows: groups A and B received Tween 80 (2%) solution orally; group C, 10 mg.kg$^{-1}$ of glibenclamide; groups D, E and F, 50, 100 and 200 mg.kg$^{-1}$ of the extract respectively. A week later (day 0), basal glycaemia was determined (74.3 ± 3.7) followed by alloxan challenge (170 mg.kg$^{-1}$) intravenously with the exception of group A. Blood was collected at days 0, 3, 5, 10 and 15 and analyzed. Inflammatory activity was evaluated by C - reactive protein (CRP). Superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (TBARS) were used to assess oxidative activity. Results: Blood glucose in the extract treated recorded maximum increase of 174.1 ± 7.7, 165.0 ± 6.7 and 166.4 ± 5.8 (50, 100 and 200 mg.kg$^{-1}$) at day 3 that was significantly less compared to the diabetic control, followed by progressive decrease in glucose level to baseline glycaemia. The tissue morphology in the extract treated showed the beta cells to be devoid of pathological changes. There was marked decrease in CRP concentration in the extract/glibenclamide treated compared to the diabetic control. The hepatic SOD and CAT levels showed recovery in the treated groups compared to diabetic control. The hepatic TBARS which increased after alloxan challenge decreased appreciably in the treated. Conclusion: SJ root showed to have protective effect on beta cells against alloxan induced diabetes.

**Keywords:** *Sphenocentrum jollyanum*, protection, beta cells.

## 1 Introduction

Diabetes is a heterogeneous metabolic disorder with severe consequences on health. It is a disease with different etiologies characterized by imbalance in blood sugar homeostasis which could be caused by either genetic or feeding habit (O'BRIEN and GRANNER, 1996). The major metabolic change caused by diabetes is the formation of oxygen free radicals that leads to oxidative stress (MOHAMED, BIERHAUS, SCHIEKOFER et al., 1999). In oxidative condition there is alteration in the endogenous free radical scavenging mechanisms which results to ineffective scavenging of Reactive Oxygen Species (ROS) from glucose glycation and auto-oxidation (OBERLEY, 1988; LATHA and PARI, 2004). Accordingly, there is the suppression of anti oxidant defenses. Therefore, anti-diabetic compound with anti-inflammatory and anti oxidant properties will be a good prophylaxis to diabetes.

Since the ancient times, plants have been used to manage diabetes. It was not until recently that herbal formulation for the development of anti-diabetic drugs have begun to attract a lot of interest following scientific validation of their anti diabetic activities.

*Sphenocentrum jollyanum* (SJ) Pierre (Menispermaceae) is a tiny shrub, an undergrowth of dense forest. It grows widely along the west coast of Africa with expanse from Sierra Leone through Nigeria to Cameroon (DALZIEL, 1955; IWU, 1993). It is reputed for use in dressing wounds particularly chronic wounds, feverish conditions and cough as well as being an aphrodisiac. It is also said to be used in the management of diabetic cases. Scientific investigations showed the plant to have anti inflammatory (MBAKA, ADEYEMI, NORONHA et al., 2006), anti tumour and anti oxidant properties (NIA, PAPER, ESSIEN et al., 2004). Its antidiabetic properties have equally been validated (MBAKA, ADEYEMI, NORONHA et al., 2009). The decision to explore the prophylactic, anti-inflammatory and anti oxidative role of SJ was based on the current context that diabetes is a disease with no known cure (WATKINS, 2003). Since free radicals play important role in diabetic predisposition (PUNITHA, RAJENDRAN, SHIRWAIKAR et al., 2005), it was therefore conceivable to investigate the protective and antioxidant effect of SJ on rabbits challenged with alloxan monohydrate.

## 2 Material and methods

### *2.1 Plant material*

Fresh roots of *S. jollyanum* were obtained in the wild from a farm land located in Ijebu-Oru community, Ogun State, Nigeria. The collection was in the month of November, and washed with tap water before being dried under the sunlight. It was authenticated by a taxonomist, Dr. O. A. Ugbogu, Chief Research Officer at the Forest Research Institute of Nigeria (FRIN) where voucher specimen of the plant has been deposited in the herbarium (FHI/108203).

### *2.2 Preparation of plant ethanol extract*

The dried roots having been chopped into pieces and dried at an ambient temperature (35-37 °C), were subjected to size reduction to a coarse powder with an electric grinder. The powder (2280 g), divided into three batches, was placed in a Soxhlet extractor and extracted with absolute ethanol, three cycles for a batch, lasting about 48 hours. The extracted material was filtered with Whatman filter paper No. 4. The filtrate obtained was dried *in vacuo* between 40-48 °C The yield about 67 g (2.94% w/w) was stored in a refrigerator (4 °C) till it was needed.

### *2.3 Animals*

Healthy adult rabbits of either sex weighing between 1.5-1.8 kg were obtained from the Animal House, University of Ibadan, Oyo State, Nigeria. Having certified their health conditions, they were kept in aluminum cages under natural light and dark cycle at the temperature of 26 ± 5 °C in the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. They were fed with standard rabbit pellets from Livestock Feeds PLC, Lagos and given water ad libitum. The use of the animals and the experimental protocol was in strict compliance with the standard guide lines on the use and care of experimental animals.

The animals were weighed before the alloxan challenge (day 0) and on days; 3, 5, 10 and 15.

### *2.4 Prophylactic study*

Rabbits randomly divided into 6 groups (8 in each), received oral treatment as follows: groups A and B received 1.7 mL of Tween 80 (2%) solution orally only; group C received 10 $mg.kg^{-1}$ of glibenclamide per day; groups D, E and F received 50, 100 and 200 $mg.kg^{-1}$ of the extract respectively by gavages which was dispersed in 2% of Tween 80 solution. A week later (day 0), basal glycaemia was determined after over night fast followed by alloxan monohydrate challenge at 170 $mg.kg^{-1}$ intravenously (i.v) as 150 $mg.kg^{-1}$ (ABDEL-HASSAN, ABDEL-BARRY and MOHAMMEDA, 2000) could not produce hyperglycaemia in the animals. Blood was collected at days 0, 3, 5, 10 and 15 and analyzed by glucose oxidase method.

### *2.5 C-reactive protein (Latex) high sensitive assay*

Roche/Hitachi/917/MODULAR P analyzers© was used to evaluate high sensitive C-reactive protein (hsCRP). Blood serum collected was centrifuged at 4000 rpm for 10 minutes and measured using photometrical method through the principle of particle-enhanced immunoturbidmetric assay (KUSHNER, GEWURZ and BENSON, 1981; EDA, KAUFMANN, ROOS et al., 1998).

### *2.6 Oxidative activity assessment*

The oxidative activity assessment was conducted in two batches, at day 3 and day 15. In the first batch, after overnight fast and blood collection, half of the animal population randomly selected from each group was sacrificed while the other half was sacrificed at the end of the study. The hepatic tissue harvested were homogenized and used for the assays.

### *2.7 Superoxide dismutase assay*

Superoxide dismutase (SOD) was assayed utilizing the technique of Kakkar, Das and Viswanathan (1984). A single unit of the enzyme was expressed as 50% inhibition of Nitroblue Tetrazolium (NBT) reduction/min/mg protein which was measured spectrophotometrically at 420 nm.

### *2.8 Catalase assay*

Catalase (CAT) was assayed colorimetrically at 620 nm and expressed as µmoles of $H_2O_2$ consumed/min/mg protein (RUKKUMANI, ARUNA, VARMA et al., 2004). The hepatic tissue was homogenized in isotonic buffer (pH 7.4). The homogenate was centrifuged at 1000 rpm for 10 minutes. The reaction mixture contained 1.0 mL of 0.01 M (pH 7.0) phosphate buffers which was added to 0.1 mL of tissue homogenate and 0.4 of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratios).

### *2.9 Estimation of lipid peroxidation*

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) were measured by the method of Niehaus and Samuelsson (RUKKUMANI, ARUNA, VARMA et al., 2004) and expressed as $nmol.mL^{-1}$. In brief, 0.1 mL of hepatic tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.3%, 0.25N HCL and 15% trichloroacetic acid) and placed in water bath for 10 minutes at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535 nm.

### *2.10 Tissue histology*

The pancreatic tissue was fixed in Bouin's fluid before being processed and stained with aldehyde fuschsin which colours the beta cells purple.

### *2.11 Phytochemical screening*

Phytochemical screening of the extract for the presence of secondary metabolites was performed using the following reagents and chemicals: alkaloids with Mayer's and Dragendorffs reagents (FARNSWORTH, 1966; HARBORNE, 1998), flavonoids with the use of Mg and HCl (SILVA, LEE and KINGHORN, 1993; HOUGHTON and RAMAN, 1998), tannins with 1% gelatin and 5% ferric chloride solution, and saponins with ability to produce suds (HOUGHTON and RAMAN, 1998). Liebermann-Buchard test consisting of a mixture of glacial acetic acid and sulphuric acid (19:1) was used to differentiate the terpenoids and steroidal compound (FARNSWORTH, 1966).

### *2.12 Acute toxicity study*

Mice randomly grouped (8 per group) received different doses (0.5, 1, 2, 4, 8 and 16 $g.kg^{-1}$) of the extract administered

by gavages. The doses were prepared by dispersing 16 g of the gel with 7 mL Tween 80 (2%) solution in a 100 mL beaker and transferred to a 20 mL volumetric flask. The beaker was thoroughly rinsed with the Tween 80 solution; the content added to the volumetric flask and the volume made to mark with the Tween 80 solution. The animals were observed continuously for the first 4 hours and then for each hour for the next 12 hours, followed by 6 hourly intervals for the next 56 hours (72 hours observations) to observe any death or changes in general behavior and other physiological activities (SHAH, GARG and GARG, 1997; BÜRGER, FISCHER, CORDENUNZZI et al., 2005).

### *2.13 Statistical analysis*

All values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analyzed using the Student's *t*-test in which $p < 0.05$ was considered significant. The difference of two means at 95 and 99% confidence levels were used for the analysis of the two sample sizes (days 3 and 15).

## 3 Results

### *3.1 Variation of body weight*

The body weight changes of the control and the groups that received extract /glibenclamide are shown in Figure 1. A reduction in body weight was observed in the animals at day 3 after alloxan challenge. From day 5, weight gain was recorded which showed progressive increase to the end of the experiment.

Using the difference of two means, at 95 and 99% confidence levels, no significant changes were observed between the values obtained at days 3 and 15.

### *3.2 Prophylactic study*

Figure 2 showed the anti-hyperglycaemic effect of SJ root extract on rabbits challenged with 170 mg.kg$^{-1}$ lethal dose of alloxan after a week of daily administration of the extract. The influence of alloxan led to increase in glycaemia. Maximum increase occurred at day 3 with glucose levels of 174.1 ± 7.7, 165.0 ± 6.7 and 166.4 ± 5.8 for the doses of 50, 100 and 200 mg.kg$^{-1}$ respectively while glibenclamide treated increased to 191.3 ± 2.5. At day 5 however, marked decrease in blood glucose level occurred with percentage decrease of 20.9 (137.8 ± 11.2), 32.2 (111.8 ± 8.1) and 34.1 (109.7 ± 10.2) for the three doses (50, 100 and 200 mg.kg$^{-1}$ respectively) that continued to the last day of the treatment. Similar decrease also occurred in glibenclamide treated.

In Figure 3, the extract groups (50, 100 and 200 mg.kg$^{-1}$ respectively) showed significant increase in CRP concentration at day 3 with percentage increase of 62.2, 42.6 and 46.3% but decreased appreciably at day 15 of treatment to 9.2, 5.2 and 4.7% respectively. Similarly, glibenclamide treated showed initial rise of 50.8% at day 3 that decreased to 6.1% at day 15.

The alloxan challenge affected the homeostasis of SOD. Figure 4 showed decrease in SOD level with maximum percentage decrease of 43.1, 35.9 and 31.3 observed at day 3 in the extract groups at the doses of 50, 100 and 200 mg.kg$^{-1}$ and 31.6% in glibenclamide group. At day 15, however, appreciable increase in SOD activity was observed in the treated groups with extract treated exhibiting dose dependent recovery.

Figure 5 showed the effect on CAT which equally recorded decrease in activity. In a mode similar to SOD, maximum decrease was recorded at day 3. The activity of CAT increased significantly ($p < 0.05$) at day 15 in the treated groups with the rate of recovery comparably higher than SOD activity.

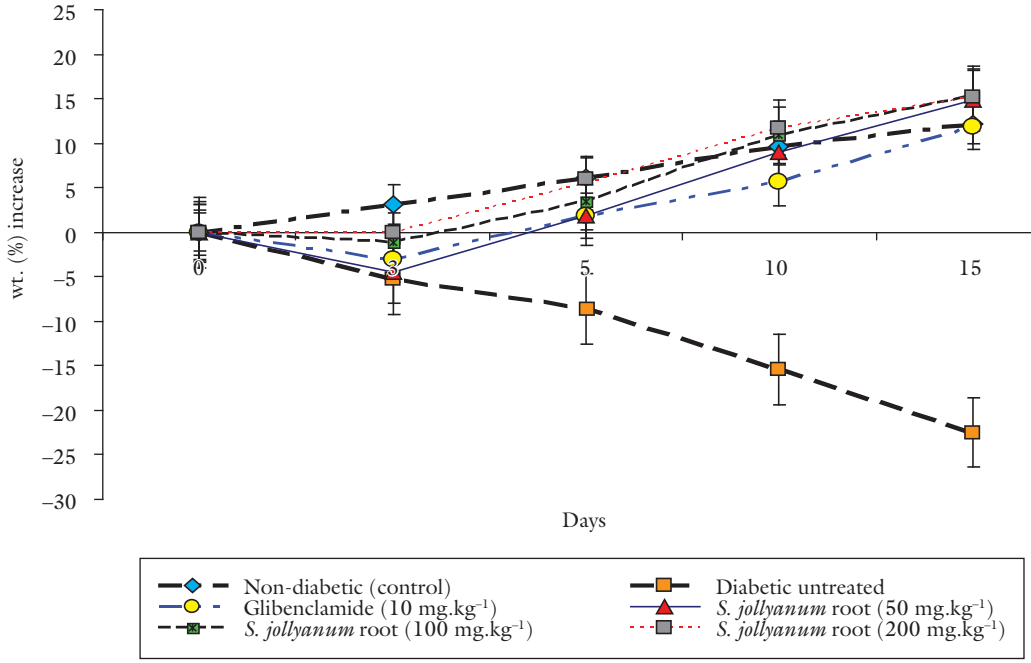


**Figure 1.** Weight increase in the treated animals.

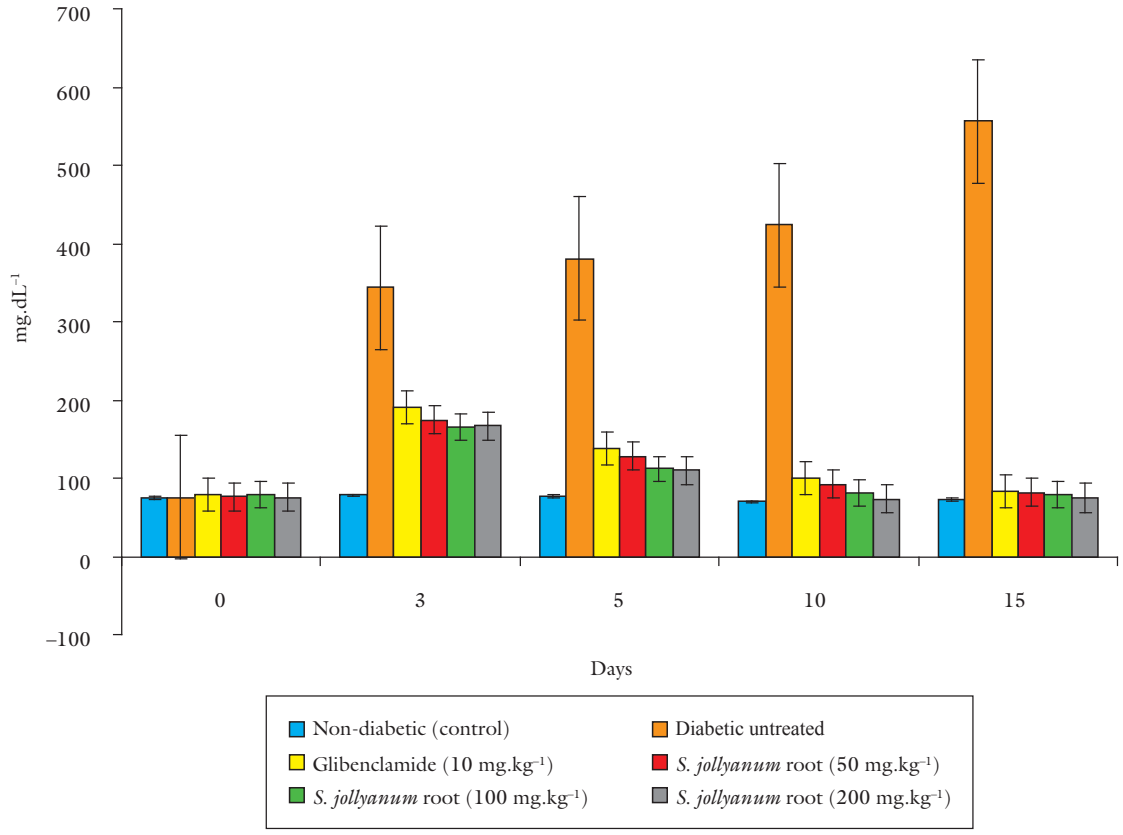


**Figure 2.** Prophylactic activity of SJ root. Values represent mean ± SEM (n = 8).

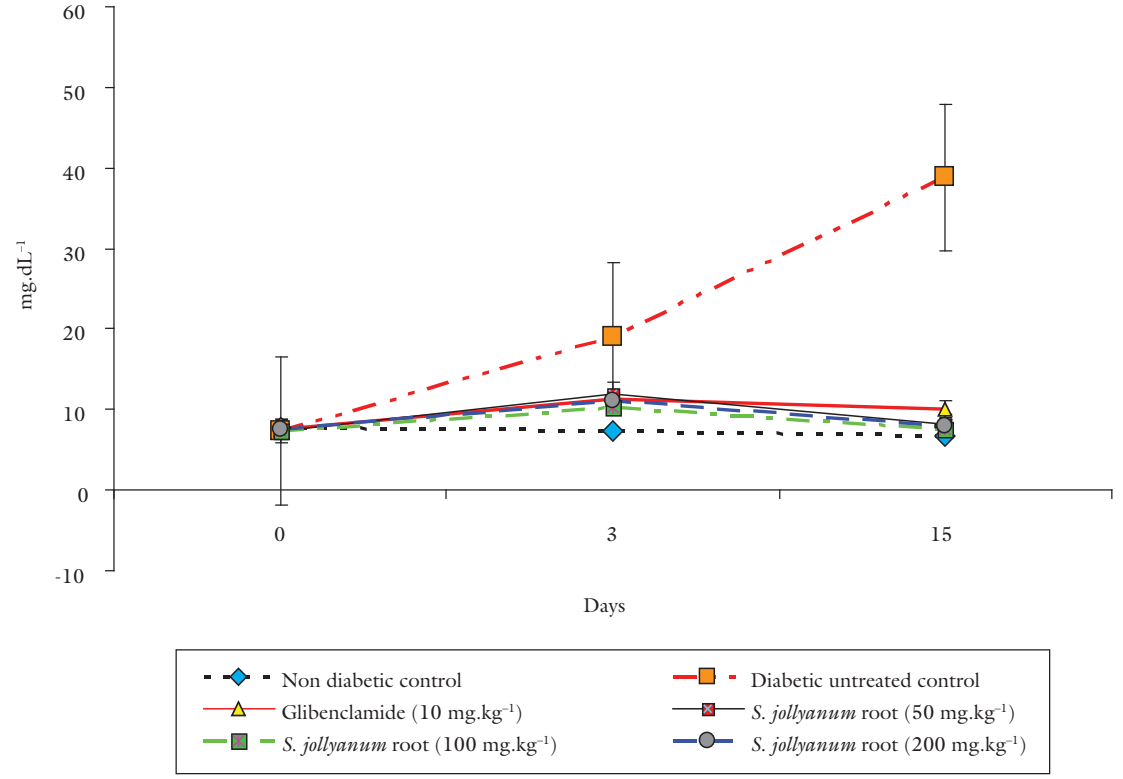


**Figure 3.** Activity of the root extract of SJ on C-reactive protein. Values represent mean ± SEM (n = 8).

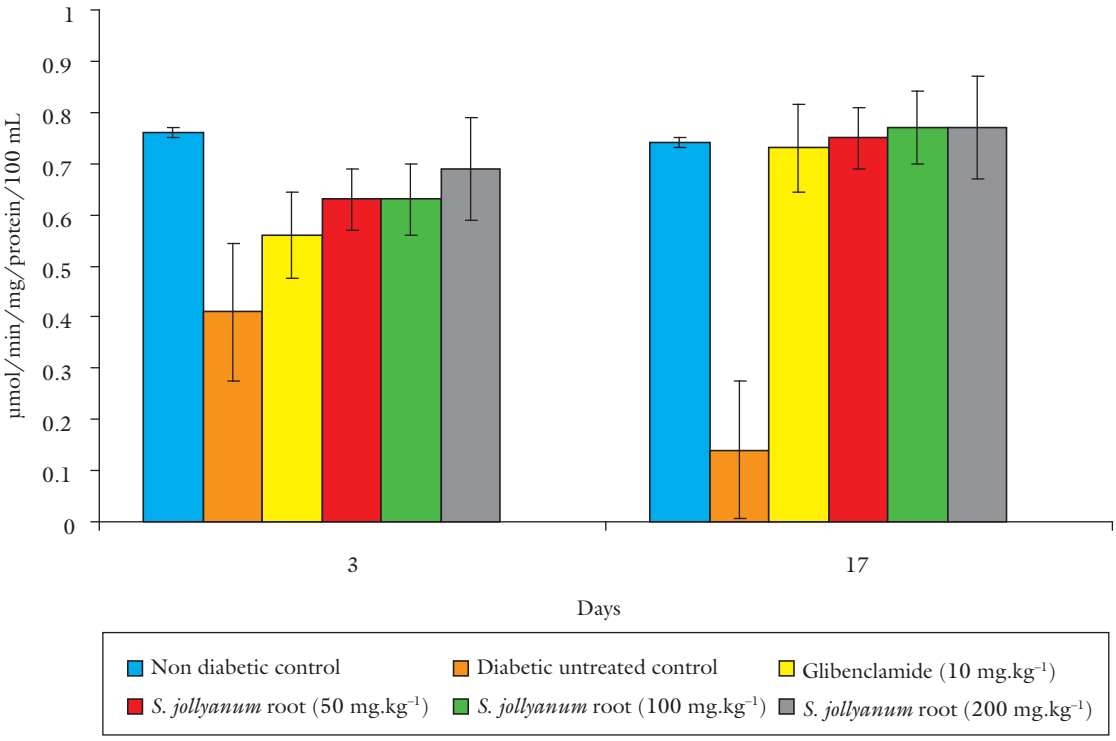


**Figure 4.** Activity of SOD in SJ root prophylactic study. Values represent mean ± SEM (n = 8).

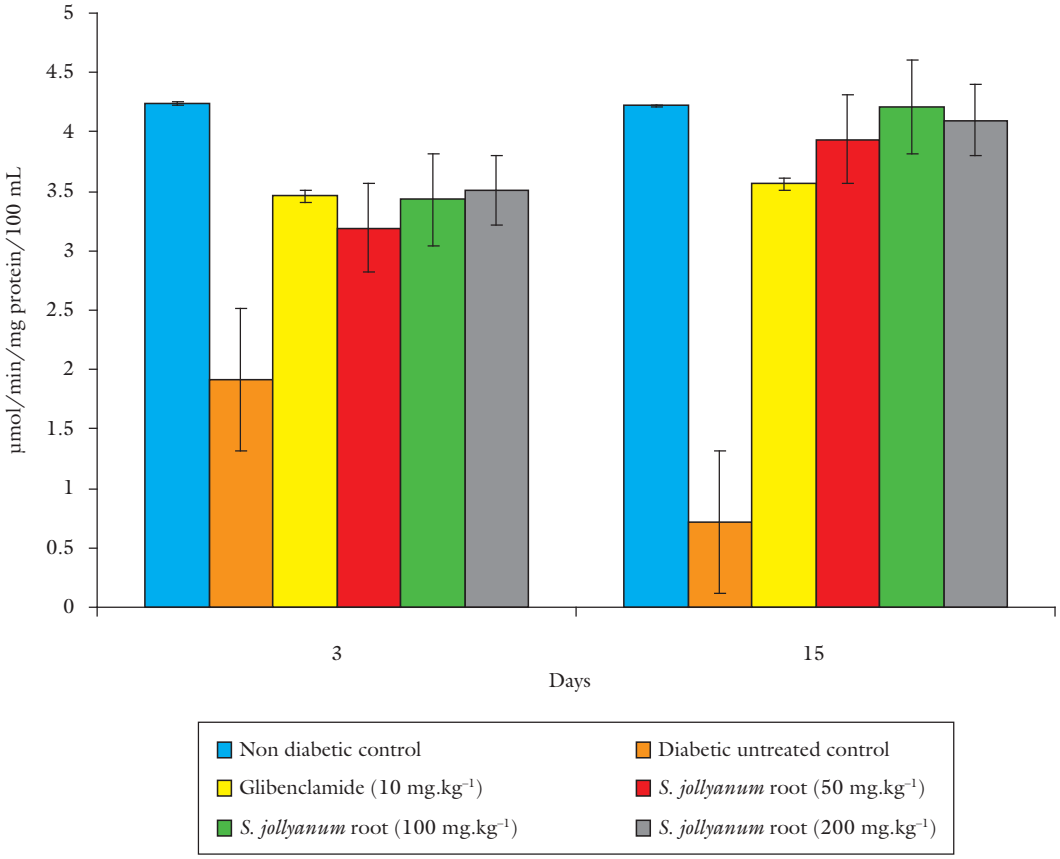


**Figura 5.** Activity of Catalase in SJ root prophylactic study. Values represent mean ± SEM (n = 8).

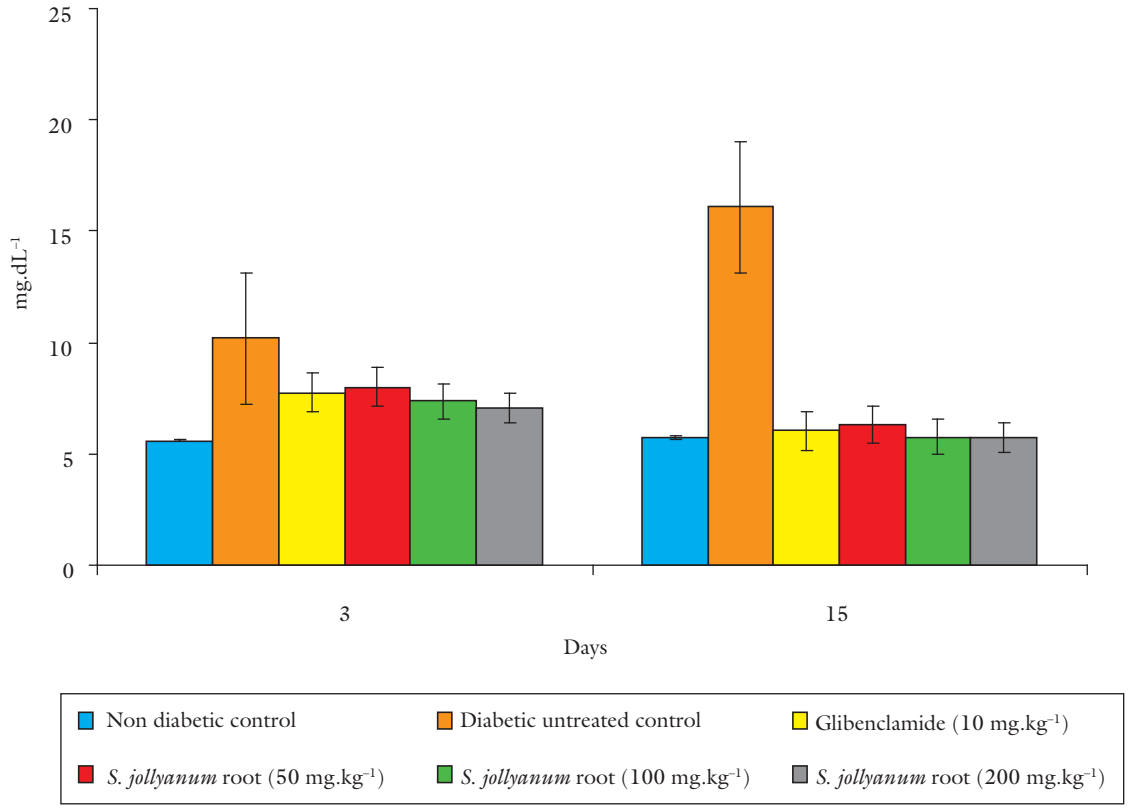


**Figure 6.** TBARS assessment in prophylactic study. Values represent mean ± SEM (n = 8).

The TBARS evaluation showed increase in activity following alloxan challenge. As observed in Figure 6, maximum increase in peroxidative activity occurred at day 3 with extract groups exhibiting percentage increase of 28.3, 38.5 and 32.2 (50, 100 and 200 mg.kg$^{-1}$ respectively) and glibenclamide group 31.9%. The TBARS level showed progressive decrease following treatment with extract and glibenclamide. At day 15, the extract groups (50, 100 and 200 mg.kg$^{-1}$) decreased to 19.6, 14.8 and 13.9% while glibenclamide group decreased to 18.6%.

### *3.3 Histopathology of pancreatic tissue*

In non diabetic specimen (Figure 7), the aldehyde fuschsin stain demonstrated the morphological features of beta cells. The stain highlighted intact beta cells arrangement in situ that was devoid of deep nuclear staining. In the photomicrograph of extract treated (Figure 8), no pathological changes occurred while mild pyknotic changes were observed at some peripheral cells in the glibenclamide treated (Figure 9). In diabetic control (Figure 10), the islet organization showed aggregate of degenerated cells that formed clump of shrunken mass of amorphous eosinophilia with a halo around it. The exocrine pancreatic acinar, ducts and connective tissue surrounding the islet cells showed normal appearance.

### *3.4 Phytochemical screening*

The active compounds found in the extract include; alkaloids, saponins, terpenoids, anthraquinones, flavonoids and tannins.

### *3.5 Acute toxicity test*

There was 100% mortality at the extract dose of 16g while no death occurred in the animals that received 4 g.kg$^{-1}$ bwt and less. The median acute toxicity ($LD_{50}$) of the extract was determined to be 9.5 g.kg$^{-1}$ bwt.

## 4 Discussion

The body weight decrease observed at day 3 might be due to the effect of alloxan challenge. The progressive weight gain recorded from day 5 suggested that the extract apart from suppressing alloxan activity may have equally improved appetite to enhance eating.

The choice of SJ in this study was because different parts of the plant have been reported to have varied pharmacodynamic effect that include anti-inflammatory (MOODY, ROBERTS, CONNOLLY et al., 2006) and anti-oxidant (NIA, PAPER, ESSIEN et al., 2004) properties. Alloxan–induced animal model was considered the right choice because it is known to be specifically cytotoxic to the pancreatic beta cells (HO, CHEN and BRAY, 1999; SARAVANAN and PARI, 2005). More so, the structure of alloxan is very similar to glucose hence its selective transport via glucose transport protein (GLUT-2 transporters) by the pancreatic islets (GORUS, MALAISSE and PIPELEERS, 1982). Alloxan has also been reported to produce free radicals during its metabolic activities while pancreatic islets are known to have lower antioxidant defense enzymes (HEIKKILA, WINSTON and COHEN, 1976; LENZEN, DRINKGERN and TIEDGE, 1996) making them more prone to oxidative damage.

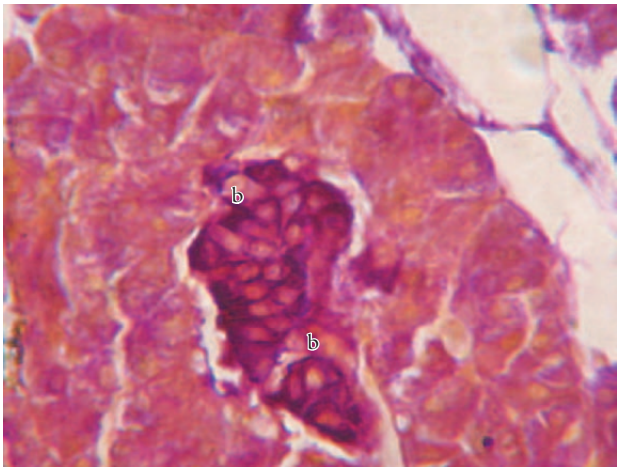


**Figure 7.** Aldehyde fuschsin stain of normal pancreatic tissue indicating beta cells (b) in light purple. Mag. ×400.

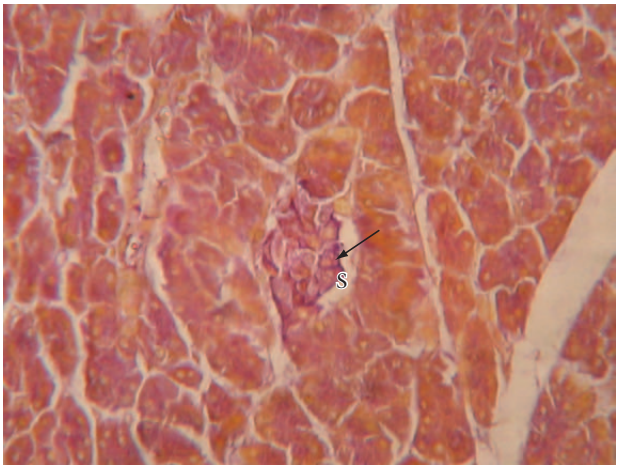


**Figure 9.** Treatment with glibenclamide: Arrow shows partial pyknotic beta cells and vacuoles (s). mag. ×400.

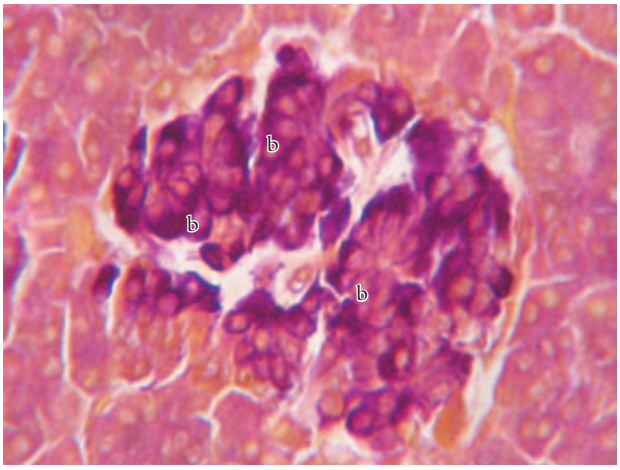


**Figure 8.** The beta cells (b) of extract treated showed no morphological changes after alloxan challenge. Mag. ×400.

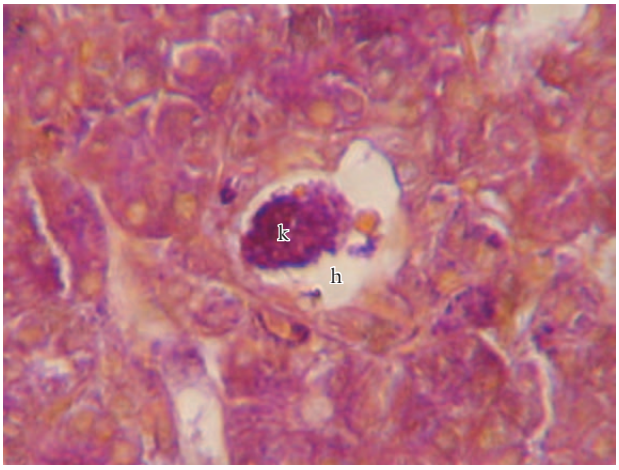


**Figure 10.** Diabetic untreated specimen showing necrotic beta cells (k) and a halo (h) around it. Mag. ×400.

In this study, SJ root extract inhibited diabetic response following alloxan challenge. Although the extract treated animals exhibited initial glycaemic increase at day 3, the rise was below diabetic index that decreased appreciably at day 5 and progressively to the last day of treatment in a dose dependent manner. It was apparent that SJ protected the beta cells against the cytotoxic effect of alloxan as was evident from the tissue morphology of the extract treated in which the photomicrograph showed the beta cells in the islet organization to be devoid of pathologic changes. The initial rise in glycaemia might be due to delayed insulin response at the acute phase activity. Studies conducted earlier on other plants have put forward similar explanation (LATHA and PARI, 2003, 2004; KASIVISWANATH, RAMESH and KUMAR, 2005).

Inflammatory processes are characterized by oxidative stress capable of generating ROS (DROGE, 2002). CRP, an inflammatory biomarker, increases rapidly during inflammatory process (PEARSON, MENSAH, ALEXANDER et al., 2003). The rise in the level of hsCRP observed at day 3 showed signs of inflammatory activity. The ability of the extract to inhibit inflammatory activity was an essential trigger that led to the suppression of superoxide radicals generated by the inflammation-mediated action of alloxan.

Impairment in normal stimulatory activity of insulin lowers the potentials of glucose utilization by the tissues which results in the formation of glycation and increased generation of oxygen free radicals (OBERLEY, 1988). Studies have shown SOD and CAT to be key antioxidant enzymes involved in the direct elimination of ROS activity (HALLIWELL and GUTTERIDGE, 1985). SOD, a major intracellular antioxidant enzyme inactivates superoxide anion reducing it to hydrogen peroxide and oxygen. CAT on the other hand, working closely with SOD, converts $H_2O_2$ into $H_2O$ and $O_2$ hence diminishes their toxic effect (BAYNES, 1995). It has been suggested that the accumulation of oxygen free radicals in hepatic tissue could reduce SOD and CAT activities (PUNITHA, RAJENDRAN, SHIRWAIKAR et al., 2005). The observance of initial decrease in SOD and CAT activities in our study were likely to be connected with oxidative stress caused by the lethal dose effect of alloxan. Treatment with SJ led to progressive increase in SOD and CAT activities which suggested decrease in the level of

reactive oxygen free radicals and improved hepatic anti-oxidant enzymes activity.

It had been reported that elevated levels of toxic oxidants in diabetes are due in part to glucose oxidation and lipid peroxidation (FEILLET-COUDRAY, ROCK, COUDRAY et al., 1999). Free oxygen radicals generated during lipid peroxidation contribute to impaired membrane functions by decreasing membrane fluidity associated with reperfusion of ischemic tissue (STARK, 2005). Increase in hepatic TBARS has been reported in alloxan induced diabetic rats (BAVARVA and NARASIMBACHARYA, 2008). In this study, we observed initial rise in TBARS following alloxan challenge. In SJ and glibenclamide treated, the TBARS decreased to near normal values with the extract treated exhibiting more effective decrease. This might be due to the fact that SJ is rich in flavonoids and diterpenoids (MOODY, ROBERTS, CONNOLLY et al., 2006) which are well known anti-oxidants (HAYASHI, KAWASKI, MIWA et al., 1990) that scavenge free radicals and protect tissue from lipid peroxidation.

## 5 Conclusion

SJ root extract has shown to have protective effect on beta cells against alloxan induced diabetes. There is a strong indication that the beta cells protection against oxidative damage may have been influenced by the anti-inflammatory and anti-oxidative activities of the plant.

**Acknowledgements:** The authors wish to thank Omolere Aye Medical Herbalist Society, Oru, Ijebu, Ogun State, Nigeria and Chief (Dr.) Adeyemi Adebambo for assisting with the plant collection.

## References

ABDEL-HASSAN, IS., ABDEL-BARRY, JA. and MOHAMMEDA, ST. The hypoglycaemic and antihyperglycaemic effect of *Citrullus cocoynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. *Journal of Ethnopharmacology*, 2000, vol. 71, no. 1-2, p. 325-330.

BAVARVA, JH. and NARASIMHACHARYA, AVRL. Antihyperglycaemic and hypolipidemic effects of *Costus speciosus* in alloxan induced diabetic rats. *Phytotherapia Research*, 2008, vol. 22, p. 620-626.

BAYNES, JW. Reactive oxygen in the aetiology and complications of diabetes. In: IOANNIDES, C. and FATT, PR., ed. *Drug, diet and disease*: mechanistic approach to diabetes. Hertfordshire: Ellis Horwood, 1995. vol. 2, p. 203-231.

BÜRGER, C., FISCHER, DR., CORDENUNZZI, DA., BATSCHAUER DE BORBA, AP., FILHO, VC. and SOARES DOS SANTOS, AR. Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (Acmela brasilinsis) (Asteraceae) in mice. *Journal of Pharmaceutical Sciences*, 2005, vol. 8, no. 2, p. 370-373.

DALZIEL, JM. *The useful plants of West Africa*. London: Crown agents for overseas governments and administration, 1955. p. 15.

DROGE, W. Free radicals in the physiological control of cell function. *Physiological Review*, 2002, vol. 82, p. 47-95.

EDA, S., KAUFMANN, J., ROOS, W. and POHL, S. Development of a new microparticle-enhanced turbidimetric assay for C- reactive protein with superior features in sensitivity and dynamic range. *Journal of Clinical Laboratory Analysis*, 1998, vol. 12, no. 3, p. 137-144.

FARNSWORTH, RN. Review on biological and phytochemical screening of plants. *Journal of Pharmacological Sciences*, 1966, vol. 55, p. 225-276.

FEILLET-COUDRAY, C., ROCK, E., COUDRAY, C., GRZELKOWSKA, K., AZAIS-BRAESCO, V., DARDEVET, D. and MAZUR, A. Lipid peroxidation and antioxidant status in experimental diabetes. *Clinica Chimica Acta*, 1999, vol. 284, p. 31-34.

GORUS, FK., MALAISSE, WJ. and PIPELEERS, DG. Selective uptake of alloxan by pancreatic beta cells. *Biochemical Journal*, 1982, vol. 208, p. 513-515.

HALLIWELL, B. and GUTTERIDGE, JMC. Free radicals and toxicology. *Free Radicals in Biology and Medicine*, 1985, p. 1-27.

HARBORNE, JB. Methods of extraction and isolation. In: *Phytochemical methods*. London: Chapman and Hall, 1998. p. 60-66.

HAYASHI, T., KAWASKI, M., MIWA, Y., TAGA, T. and MORITA, N. Antiviral agents of plant origin III. Scopadulin, a novel tetracyclic diterpene from *Scoparia dulcis* L. *Chemical and Pharmaceutical Bulletin*, 1990, vol. 38, p. 945-947.

HEIKKILA, RE., WINSTON, B. and COHEN, G. Alloxan-induced diabetes-evidence for hydroxyl radical as a cytotoxic intermediate. *Biochemical Pharmacology*, 1976, vol. 25, p. 1085-1092.

HO, E., CHEN, G. and BRAY, TM. Supplementation of N-acetylcysteine inhibits NFkB activation and protects against alloxan-induced diabetes in CD-1 mice. *FASEB Journals*, 1999, vol. 13, no. 13, p. 1845-1854.

HOUGHTON, PJ. and RAMAN, A. *Laboratory handbook for fractionation of natural extracts*. London: Chapman and Hall, 1998. p. 199.

IWU, MM. *Handbook of African medicinal plants*. CRC Press Inc, 1993. p. 239.

KAKKAR, P., DAS, B. and VISWANATHAN, PN. A modified spectrophotometric assay of superoxide dismutase (SOD). *Indian Journal of Biochemistry and Biophysics*, 1984, vol. 21, no. 130-132.

KASIVISWANATH, R., RAMESH, A. and KUMAR, KE. Hypoglycaemic and antihyperglycaemic effect of *Gmelina asiatica* Linn. in alloxan induced diabetic rats. *Biological and Pharmaceutical Bulletin*, 2005, vol. 28, p. 729-732.

KUSHNER, I., GEWURZ, H. and BENSON, MD. C-reactive protein and the acute-phase response. *Journal of Laboratory and Clinical Medicine*, 1981, vol. 97, p. 739-749.

LATHA M. and PARI, L. Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. *Clinical and Experimental Pharmacology and Physiology*, 2003, vol. 30 no. 1-2, p. 38-43.

LATHA, M. and PARI, L. Effect of an aqueous extract of *Scoparia dulcis* on blood glucose, plasma insulin and some poly pathway enzymes in experimental rat diabetes. *Brazilian Journal of Medical and Biological Research*, 2004, vol. 37, no. 4, p. 577-586.

LENZEN, S., DRINKGERN, J. and TIEDGE, M. Low anti oxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radical Biology and Medicine*, 1996, vol. 20, p. 463-466.

MBAKA, GO., ADEYEMI, OO., NORONHA, CC., ANUNOBI, CC. and OKANLAWON, AO. Anti- hyperglycaemic and hypoglycaemic effects of ethanol root extract of *Sphenocentrum jollyanum* in normal and alloxan-induced diabetic rabbits. *Brazilian Journal of Morphological Sciences*, 2009, vol. 26 no. 2, p. 123-127.

MOHAMED, AK., BIERHAUS, A., SCHIEKOFER, S., TRITSCHLET, H., ZIEGLER, H. and NAWROTH, PP. The role of oxidative stress and NF (B) activation in late diabetic complications. *Biofactors*, 1999, vol. 10, p. 175-179.

MOODY, JO., ROBERTS, VA., CONNOLLY, JD. and HOUGHTON, PJ. Anti- inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). *Journal of Ethnopharmacology*, 2006, vol. 104, no. 1-2, p. 87-91.

NIA, R., PAPER, DH., ESSIEN, EE., LYADI, KC., BASSEY, AIL., ANTAI, AB. and FRANZ, G. Evaluation of the anti-oxidant and anti-angiogenic effects of *Sphenocentrum jollyanum* Pierre. *African Journal of Biomedical Research*, 2004, vol. 7, p. 129-132.

O'BRIEN, RM. and GRANNER, DK. Regulation of gene expression by insulin. *Physiological Review*, 1996, vol. 6, p. 1109-1161.

OBERLEY, LW. Free radicals and diabetes. *Free Radical Biology and Medicine*. 1988, vol. 5, p. 113-124.

PEARSON, TA., MENSAH, GA., ALEXANDER, RW., ANDERSON, JL., CANNON, RO., CRIQUI, M., YAZID, YF.,FORTMANN, SP., HONG, Y., MYERS, GL., RIFAI, N., SMITH, SC., TAUBERT, K., RUSSEL, PT. and VINICOR, F. Markers of inflammation and cardiovascular disease. Application to clinical and public health practice. A statement for healthcare professionals from the centres for disease control and prevention and American heart association. *Circulation*, 2003, vol. 107, p. 499-511.

PUNITHA, ISR., RAJENDRAN, K., SHIRWAIKAR, A. and SHIRWAIKAR, A. Alcoholic stem extract of *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. *eCAM*, 2005, vol. 10, p. 1-7.

RUKKUMANI, R., ARUNA, K., VARMA, OS., RAJASEKARAN, KN. and MENON, VP. Comparative effects of curcumin and analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacological Sciences*, 2004, vol. 7, no. 2, p. 274-283.

SARAVANAN, R. and PARI, L. Antihyperlipidemic and antiperoxidative effect of Diasulin, a polyherbal formulation in alloxan induced hyperglycaemic rats. *BMC Complementary and Alternative Medicine*, 2005, vol. 5, no. 10, p. 1186-1472.

SHAH, AMA., GARG, SK. and GARG, KM. Subacute toxicity studies on Pendimethalin in rats. *Indian Journal of Pharmacology*, 1997, vol. 29, p. 322-324.

SILVA, LG., LEE, IS. and KINGHORN, DA. Special problems with the extraction of plants. In: CANNELL, JPR., ed. *Methods in biotechnology natural product isolation*. Totowa, New Jersey, USA: Humana Press, 1993. vol. 4, p. 329-363.

STARK, G. Functional consequences of oxidative membrane damage. *Journal of Membrane Biology*, 2005, vol. 205, p. 1-16.

WATKINS, P. *ABC of diabetes*. 5th ed. BMJ Publishing's Ltd, 2003. p. 1-6.

Received June 18, 2010
Accepted March 2, 2011