Journal of Medicinal Plants Research Vol. 6(16), pp. 3193-3198, 30 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1693
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Anti-diabetic activity of aqueous root extract of *Anacyclus pyrethrum* L. in streptozotocin-induced-diabetic rats

Chaouki Selles[1], Houria Medjdoub[2], Mohammed El Amine Dib[1]*, Meriem Zerriouh[1] and Boufeldja Tabti[1]

[1]Laboratoire des Substances Naturelles et Bioactives (LASNABIO) Département de Chimie, Faculté des Sciences, Université Aboubekr Belkaïd BP 119, Tlemcen 13000, Algérie.
[2]Département de biologie, Faculté des sciences de la nature et de la vie, Université de Mascara., Algérie.

Accepted 30 January, 2012

The present work is to study for the first time the anti-diabetic properties of aqueous extract of roots of *Anacyclus pyrethrum* L. in normal and streptozotocin (STZ)-induced diabetic rats and to achieve a primary pharmacological screening contained in the aqueous extract. A total of 20 rats including 10 diabetics and 10 normal rats were used for this study. The anti-diabetic activity of aqueous extract of roots was evaluated by using normal and STZ induced diabetic rats at a dose of 250 mg/kg *p.o* daily for 21 days. Blood glucose levels were measured using GOD-POD. Screening for major classes of phytochemical was done using standard chemical tests. Per oral administration of the aqueous extract of the roots (250 mg/kg body weight) to streptozotocin-induced diabetic rats exhibited a significant antihyperglycemic activity in STZ-induced diabetic rats, whereas in normal rats no hypoglycemic activity was observed. Phytochemical screening showed a wealth in compounds: Tannins, saponins, alkaloids, amino acids, steroids and terpenoids. Aqueous extract of roots exhibit attractive properties and can therefore, be considered a promising candidate for future application as alternative therapeutic agents, particularly in the development of anti-diabetic drugs.

Key words: *Anacyclus pyrethrum* L, anti-diabetic activity, streptozotocin, aqueous root extract, phytochemical screening.

INTRODUCTION

Diabetes is one of the most prevalent chronic diseases in the world, It is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Considerable attention has been placed on understanding the pathophysiology of diabetes mellitus because of its importance in human health. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (American Diabetes Association, 2008). Current treatment includes insulin therapy, although this provides good glycemic control, it can do little to prevent secondary complications. Besides, these drugs are associated with side-effects or diminution in response after prolonged use (Mahadeva and Subramanian, 2009). Moreover, providing modern medical healthcare across the world is still a far-off goal due to economic constraints. Thus, it is necessary that we continue to look for new and, if possible, more efficacious drugs, and the vast reserves of phytotherapy may be an ideal target (Mahadeva and Subramanian, 2009). Plants have played a significant role in maintaining human health and improving quality of life for thousands of years. In particular, herbs have been used as food and for medicinal purposes for centuries. The use of medicinal plants is increasing because of their widespread use and for their curative effects on various diseases (Bhavsar et

*Corresponding author. E-mail: a_dibdz@yahoo.fr. Tel/Fax: +213 43286530.

al., 2009). In order to discover other hypoglycaemic agents, many investigations have been performed in traditional medicine testing eventual hypoglycaemic plants (Girija et al., 2011; Meliani et al., 2011; Salahuddin et al., 2010; Kumar et al., 2010; Solati et al., 2010; Adeneye et al., 2009). *Anacyclus pyrethrum* L. (Compositae, Asteraceae), commonly known as "*African pyrethrum*" and referred to as "Tigenthast", is a perennial, procumbent herb, which is native from Northern Africa and is cultivated in the Mediterranean (Bellakhdar, 1997). The root of *A. pyrethrum* is being widely used in the local traditional medicinal treatment system for a variety of several diseases: it is used in the treatment of some rheumatic and neuralgic affection of the head, the tooth and face. A local ethnobotanical survey carried out showed its possible anti-diabetic activity (Allali et al., 2008). Previous chemical studies indicate that the plant possesses immunomodulatory properties (Bendjeddou et al., 2003; Ching et al., 2007). Everywhere in Algeria, the root is used as sternutatory, sialagogue and diaphoretic. It is regarded as a tonic to the nervous system. It is also used in respiratory infections and in the treatment of liver disease (Bellakhdar, 1997; Bendjeddou et al., 2003). In some other countries, the roots are also considered aphrodisiac and sexual stimulant. In Indian medicine, the plant is widely recognized as tonic and rejuvenator (Singh et al., 1998). It has been reported that the *A. pyrethrum* root has antibacterial and anti-inflammatory activities and is known for its insecticidal properties (Bellakhdar, 1997). However, the literature indicates that there is no scientific evidence to support the anti-diabetic effect of *A. pyrethrum* in spite of its ethnobotanical usage. The aim of the present work was evaluate for the first time the anti-diabetic activity of *A. pyrethrum* to ascertain the scientific basis for the use of this plant in the treatment of diabetes and to do the phytochemical preliminary screening of roots.

MATERIALS AND METHODS

Plant material

The roots of *A. pyrethrum* were harvested from the mounts of Tlemcen (north-west of Algeria), in April 2009. The plants collected were identified by Pr. Noury Benabadji, "Laboratory of Ecology and Ecosystem Management", University of Tlemcen (Algeria). A voucher specimen was deposited in this laboratory. Plant samples were dried in the share and conserved for future use.

Preparation of aqueous extract

Fresh roots were dried at room temperature. Aqueous extract was obtained as follows. In brief, 100 g of dried roots were cut into small bits and extracted by refluxing with distilled water for one hour. Thereafter, it was decanted and filtered with filter paper and followed by centrifugation for 30 min at 5000 rpm. The supernatant was filtered to eliminate any residues. The filtrate was dried in the oven at 40°C to make a powder yielding 2.3% (w/w). The solid residue was stored in dessicator prior use for subsequent experiments.

Phytochemical analysis

Alkaloids

Five milliliters of the crude extract were added to 2 mL of hydrochloric acid. One milliliter of Dragendroff's reagent was added to this acidic medium. An orange or red precipitation was produced which indicates the presence of alkaloids (Kokate, 2001).

Amino acids

One milliliter of the crude stock extract was added a few drops of Ninhydrin reagent. The purple colour appearance shows the presence of amino acids (Harborn, 1998).

Saponins

The crude extract solution was diluted with 20 mL of distilled water and it was agitated in a graduated cylinder for 15 min. The formation of 1 cm foam layer showed the presence of saponins (Kokate, 2001; Harborn, 1998).

Flavonoids and tannins

To one milliliter of the crude stock extract, 10 mg magnesium turnings were added into 1 mL of the filtrate, followed by the addition of 0.05 mL concentrated sulphuric acid. The presence of magenta red observed within 3 min confirmed the presence of flavonoids. The presence of blue-black precipitates resulting from the addition of ferric chloride (0.01 g/mL) reagent indicated the presence of tannins (Harborn, 1998).

Steroids and terpenoids

Screening the presence of steroids and terpenoids was performed as described by Kumar (Kumar et al., 2009). 0.2 g of extract was dissolved in 10 mL methanol and filtered. 1 mL of chloroform and 1 mL of concentrated sulphuric acid were then added into 1 mL of filtrate by the side of the tube and the presence of yellow with green fluorescence at the sulphuric acid layer indicated the presence of steroids. For the detection of terpenoids, 1 mL of acetic anhydride and 2 mL of concentrated sulphuric acid were added into 1 mL filtrate. Presence of reddish brown on interface indicated the presence of terpenoids.

Animals

Male wistar rats between 12-13 week of age and weighing 250-300g were procured from Mascara University Animal house, Algeria. The animals were under standard conditions and fed with rodent diet and water *ad libitum*.

Induction of diabetes

Diabetes was induced in rats by intravenous (*i.v*) injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, dissolved in 0.1M cold citrate buffer (pH = 4.5) (Sancheti et al., 2010). Blood glucose level was measured using GOD-POD (Kit spinreact). Blood samples were withdrawn from retro-orbital plexus under light ether

Table 1. Phytochemical prospection of aqueous extract roots of *A. pyrethrum*.

	Metabolites						
Extract	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Aqueous Root	+	-	+	+	+	+	+

1: tannins; 2: flavononols; 3: saponins; 4: terpenoids; 5: steroids; 6: alkaloids; 7: amino acids; +: presence; - : absence.

anaesthesia before. A fortnight later, diabetes was confirmed.

Acute toxicity

Acute toxicity study on *A. pyrethrum* roots extract was performed in experimental rats. Graded doses of the aqueous extract of roots (100, 250, 500 and 1000 mg/kg body weight) were administered *p.o* and the animals were observed for 2 weeks following administration (Olagbende-Dada et al., 2010). The dosage schedule was fixed as 250 mg/kg body weight/rat/day for 21 days.

Experimental protocol

The normal rats and those with hyperglycemia (blood glucose 200-360 mg/100 mL) were divided into four groups of five animals each and treated by per oral as shown below.

Group 1: Normal control given only saline (9 g/L) (NC),
Group 2: Control treated with aqueous extract at dose of 250 mg/Kg (NTAE),
Group 3: Diabetic control given only saline (9 g/L) (DC),
Group 4: Diabetic rats treated with aqueous extract at dose of 250 mg/Kg (DTAE).

Blood samples and body weight were measured at weekly intervals on days 0, 7, 14 and 21, till the end of study. Blood glucose levels were measured using GOD-POD (Kit spinreact) (Trinder, 1996). At the end of the experiment, an oral glucose tolerance (OGTT) test was practised. Animals (four groups) were loaded with glucose (3 g/kg). Blood glucose level was determined at 0, 60 and 120 min after glucose loading.

Statistical analysis

All the values of body weight, fasting blood sugar, and OGTT were expressed as mean ± SEM. Data were analyzed statistically using the student's t-test. In all cases, $p<0.05$ was used as the criterion of statistical significance.

RESULTS

Preliminary phytochemical screening

Table 1 shows the presence of various compounds such as tannins, amino acids, alkaloids, saponins steroids and terpenoids. However, flavonoids are completely absent. Through phytochemical prospecting of the extract, it was possible to determine the presence of diverse classes of secondary metabolites that show a wide variety of biological activities such as antimicrobial (Todkar et al., 2010), antioxidant (Benhammou et al., 2009) and anti-diabetic (Debbab et al., 2010).

Acute toxicity study

In the present study, all animals that were fed with amounts of 100, 250, 500 and 1000 mg/kg body weight of *A. pyrethrum* aqueous extract have reacted well after treatment and showed no signs of toxicity or behavioral changes at any doses selected until the end of study.

Changes in body weight

Figure 1, shown the change in body weight gain in control rats and control treated with aqueous extract. The body weight was decreased in diabetic rats while oral administration of root extract improves the body weight gain in STZ-induced diabetes.

Oral glucose tolerance test in normal rats (OGTT)

Oral glucose tolerance test in diabetic and normal rats showed an increasing in blood glucose level one hour after glucose administration. A significant reduction was marked in diabetic treated ($P< 0.05$) with aqueous extract 2 h (Figure 2) after treatment and suppressed the rise in blood glucose compared with standard group.

Antihyperglycaemic effect of aqueous extract

The effect of the aqueous extract of roots on the fasting blood glucose levels of both normal and diabetic rats are given in Table 2. The fasting blood glucose levels of diabetic untreated rats (Group 4) were significantly higher than those of normal untreated rats (Group 1). The extract of roots at a dosage of 250 mg/kg produced the

Table 2. Effect of 3-week treatment with aqueous extract of roots of *A. pyrethrum* on blood glucose level in STZ (50 mg/Kg ip),-induced diabetic in rats (Mean±SEM).

Group (n=4)	Treatment	Dose (mg/kg)	Blood glucose concentration (mg/dl)			
			Day 0	Day 7	Day 14	Day 21
1	NC		119.7 ± 5.1	120.0 ± 4.5	113.0 ± 8.0	97.0 ± 1.9
2	NTAE	250	107.7 ± 2.4	107.7 ± 1.0	97.7 ± 6.3	106.0 ± 1.5
3	DC		226.7 ± 2.4*	297.7 ± 2.9*	281.2 ± 3.4*	360.6 ± 4.1*
4	DTAE	250	221.0 ± 2.0	120.7 ± 1.7**	107.7 ± 1.2**	83.7 ± 1.6**

STZ single dose of 50 mg/kg *p.o* on day 0; * p < 0.05 when compared with normal control, ** p < 0.001 when compared with diabetic control.

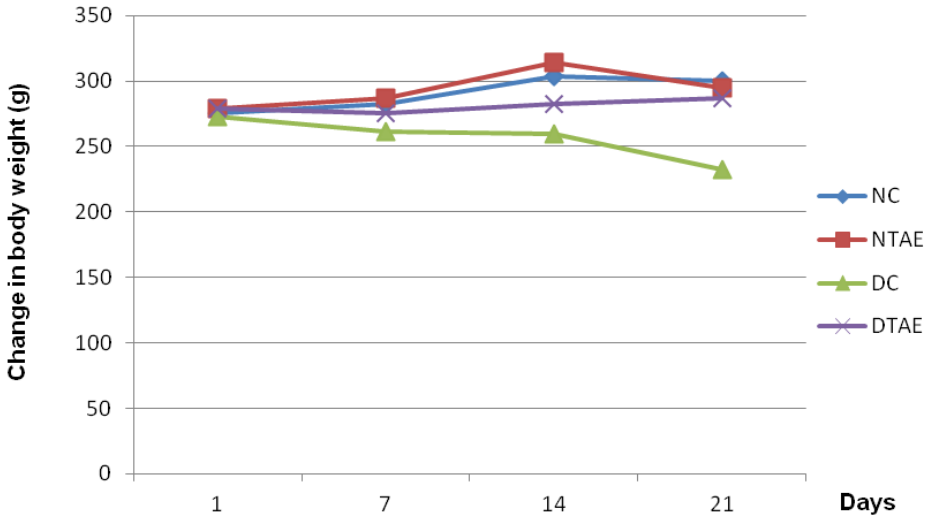

Figure 1. Change in body weight in the treatment of aqueous extract in STZ induced diabetic rats.

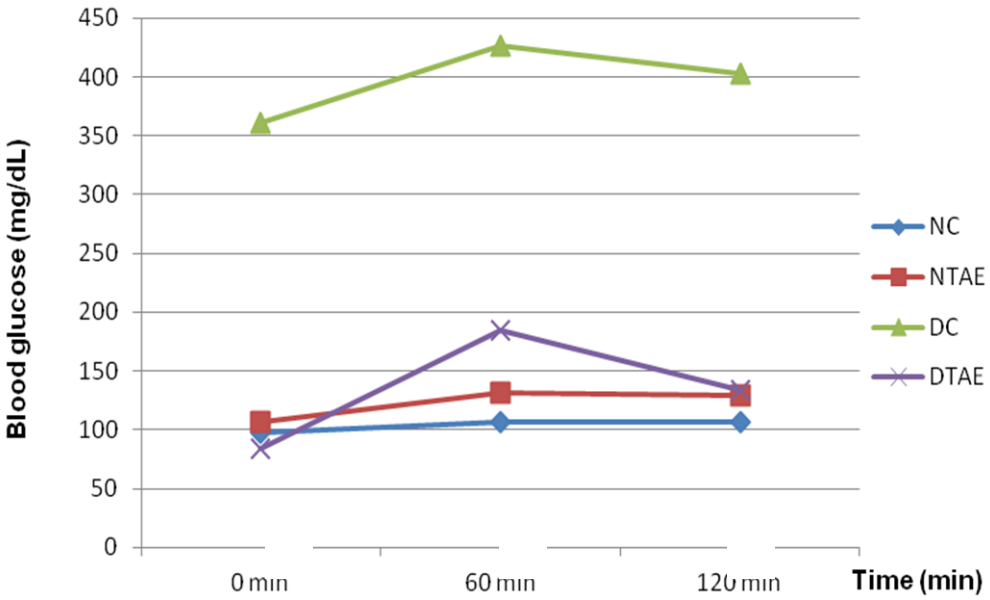

Figure 2. Effects of aqueous extract of A. pyrethrum on OGTT in normal rats (Mean±SEM).

maximum fall of 62.12% in the blood glucose levels of diabetic rats after 21 days of treatment. However any hypoglycemic effect in normal treated rats was observed.

DISCUSSION

Blood glucose is a key marker for diagnosis and prognosis of diabetes mellitus. Insulin deficiency causes drastic elevation in levels of blood glucose as a result of excessive production of endogenous glucose and also causes a drastic change in body weight (Ramachandran et al., 2011), which may be due to excessive breakdown of tissue proteins and lipids caused by insulin insufficiency. Various anti-diabetic plants and herbs are found through traditional use but their introduction into modern therapy needs testing of the compounds by modern research methodology. In the present study, oral treatment with *A. pyrethrum* roots extract appreciably lowered the level of blood glucose and improved the insulin level in STZ-induced diabetic rats. The anti-hyperglycemic effect of aqueous extract may be due to stimulatory effect on the remnant β-cells to secrete more insulin or from regenerated β-cells (Mahadeva and Subramanian, 2009). Another observation arising from this study is the effect of the aqueous extract of roots on the body weight in the treated rats. The improvement in body weight in diabetic rats treated may be due to improvement in metabolic activity of the system to maintain glucose homeostasis. These results suggested that root contained some biological principle(s) that posses insulin protective or insulin-like activity (Maqsood and Tanveer, 2009) but again this hypothesis would require experimental validation and further experiments are required to elucidate the exact mechanism of action as well as on the isolation of bioactive principles. The phytochemical screening of root revealed the presence of tannins, saponins, alkaloids, amino acids, steroids and terpenoids that are known to be bioactive antidiabetic principles (Meliani et al., 2011). Amino acids have been reported as antihyperglycemic agents, because that absolute or relative lack of insulin leads to defective amino acid/protein metabolism (Capraro et al., 2010). Phenolics are found to be effective antihyperglycemic agents (Abdelmoaty et al., 2010; Hossam et al., 2011). Alkaloids and tannins have been reported to possess hypoglycemic activity (Sharma et al., 2010; Nkirote et al., 2011). The saponin is known to elicit serum cholesterol lowering activity and may be classified as a direct hypoglycaemic agent, in contrast to the indirect agents such as the sulphonylureas that act by stimulating the pancreatic beta cells to release more insulin (Chang-yong et al., 2010; Abdel-Hassan et al., 2000).

Conclusion

It can be stated, that the aqueous root extract of *A. pyrethrum* has beneficial effects, in reducing the elevated blood glucose level of STZ-induced-diabetic rats, but has no effect on normal rats. Further studies will be focussed on determination of the mechanism(s) of action, as well as on the isolation of bioactive principles for contribute toward the development of a potent anti-diabetic drug.

ACKNOWLEDGMENTS

The authors would like to express thanks to N. Benabadji of Botanical Laboratory, Biology Department, Aboubekr Belkaïd University for identification of the vegetable matter.

REFERENCES

Abdel-Hassan IA, Abdel-Barry JA, Mohammeda ST (2000). The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. J. Ethnopharmacol., 71: 325–330.

Abdelmoaty MA, Ibrahim MA, Ahmed NS, A Abdelaziz MA (2010). Confirmatory studies on the antioxidant and antidiabetic effect of Quercetin in rats. Indian J. Clin. Biochem., 25 (2): 188-192.

Adeneye AA, Olagunju JA (2009). Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn. in Wistar rats. Biol. Med., 1: 1-10.

Allali H, Benmehdi H, Dib MA, Tabti B, Ghalem S, Benabadji N (2008). Phytotherapy of Diabetes in West Algeria. Asian J. Chem., 20 (4): 2701-2710.

American Diabetes Association (ADA) (2008). *Diagnosis and classification of diabetes mellitus*. Diabetes Care, 31: S55–S60.

Bellakhdar J (1997). La Pharmacopée Marocaine Traditionnelle: Médecine arabe ancienne et savoirs populaires - Saint –Etienne, Edit. Ibis Press, p. 177.

Bendjeddou D, Lalaoui K, Satta D (2003). Immunostimulating activity of the hot water-soluble polysaccharide extracts of *Anacyclus pyrethrum*, *Alpinia galanga* and *Citrullus colocynthis*, J. Ethnopharmacol., 88: 155.

Benhammou N, Atik Bekkara F, Kadifkova Panovska T (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus* .C. R. Chimie. 12 (9): 1259-1266.

Bhavsar SK, Singh S, Giri S, Jain MR, Santani DD (2009). Effect of saponins from *Helicteres isora* on lipid and glucose metabolism regulating genes expression. J. Ethnopharmacol., 124: 426-433.

Capraro J, Spotti P, Magni C, Scarafoni A, Duranti M (2010). Assessment of the lupin seed glucose-lowering protein intestinal absorption by using in vitro and ex vivo models. J. Agric. Food Chem., 57: 8612-8616.

Chang-yong Y, Jing W, Yuan Z, Li S, Xing J, Zhen-guo X, Nan L, Liu Z, Zhong-hua C (2010). Anti-diabetic effects of *Panax notoginseng* saponins and its major anti-hyperglycemic components. J. Ethnopharmacol., 130: 231–236.

Ching An Wu , Jia Jiu Wu, Meei Ju Tsai, Ru Yin Chen (2007). Immunomodulatory effects of a traditional Chinese medicine, Chi-Shie-Shuang-Bu-An-Shen-Tang, on BALB/c mice, J. Ethnopharmacol.,113: 300.

Debbab A, Aly AIH, Lin WH, Proksch P (2010). Bioactive Compounds from Marine Bacteria and Fungi. Microbial Biotechnol., 3(5): 544-563.

Girija K, Lakshman K, Udaya CH, Sabhay Sahi G, Divya T (2011). Anti-diabetic and anti-cholesterolemic activity of methanol extracts of three species of *Amaranthus*. Asian pac J Trop Biomed., 1(2): 133-138.

Harborn JB (1998). Phytochemical Methods. A guide to modern techniques of plant analysis. Chapman & Hall; London, p. 302.

Hossam M, Abdallah, Maha M, Salama, Enas H, Abd-elrahman, Shohda A El-Maraghy (2011). Antidiabetic activity of phenolic compounds from Pecan bark in streptozotocininduced diabetic rats. Phytochem. Letters, 4: 337–341.

Kokate CK (2001). Pharmacohnosy, 16th ed. Nirali Prakasham, Mumbai, India.
Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanabhan N, Krishnan MRV (2009). Phytochemical investigation on a tropical plant, Syzygium cumini from Kattuppalayam, Erode District, Tamil Nadu, South India. Pak. J. Nutrit., 8(1): 83-85.
Kumar S, Kumar v, Prakash Om (2010). Antidiabetic and anti-lipemic effects of *Cassia siamea* leaves extract in streptozotocin induced diabetic rats. Asian pac. J. Trop. Med., 871-873.
Mahadeva Rao US, Subramanian ES (2009). Biochemical evaluation of antihyperglycemic and antioxidative effects of *Morinda citrifolia* fruit extract studied in streptozotocin-induced diabetic rats. Med. Chem., Res., 18: 433-446.
Maqsood AA, Tanveer S (2009). A potential adjunct to insulin: *Berberis lyceum royle*. Diabetologia Croatica, 38(1): 13-18.
Meliani N, Dib MA, Allali H, TAbti B (2011). Hypoglycaemic effect of *Berberis vulgaris* L. in normal and streptozotocin-induced diabetic rats. Asian pac. J. Trop. Biomed., 1(6): 468-471.
Nkirote CK, Kathenya IJ, Michael O, Clare M, Konrad BH, Vellingiri V (2011). Antioxidant and Antidiabetic Properties of Condensed Tannins in Acetonic Extract of Selected Raw and Processed Indigenous Food Ingredients from Kenya. J. Food Sci., 76(4): C560-C567.
Olagbende-Dada SO, Ogbonnia SO, Coker HAB, Ukpo GE (2011). Blood glucose lowering effect of aqueous extract of *Graptophyllum pictum* (Linn) Griff. on alloxan-induced diabetic rats and its acute toxicity in mice. Afr. J. Biotechnol., 10(6): 1039-1043.
Ramachandran S, Rajasekaran A, Kumar KTM (2011). Antidiabetic, antihyperlipidemic and antioxidant potential of methanol extract of *Tectona grandis* flowers in streptozotocin induced diabetic rats. Asian pac. J. Trop. Med., 624-631.
Salahuddin Md, Jalalpure Sunil S (2010). Antidiabetic activity of aqueous fruit extracts of *Cucumis trigonus* Roxb. In streptozotocin-induced diabetic rats. J. Ethnopharmacol., 127: 565–567.
Sancheti S, Sancheti S, Bafna M, Seo SY (2010). Antihyperglycemic, antihyperlipidemic, and antioxidant effects of *Chaenomeles sinensis* fruit extract in streptozotocin-induced diabetic rats. Eur. Food Res. Technol., 231: 415-421.
Sharma B, Salunke R, Balomajumder C, Daniel S, Roy P (2010). Anti-diabetic potential of alkaloid rich fraction from *Capparis decidua* on diabetic mice. J. Ethnopharmacol., 127: 457-462.
Singh G, Mukherjee T (1998). Herbal aphrodisiacs: a Review, Indian Drugs, 34: 175.
Solati J, Soleimani N (2010). Antihyperglycemic and antihyperlipidemic effects of *Ziziphus vulgaris* L. onreptozocin-induced diabetic adult male Wistar rats. Acta Diabetol., 47 (Suppl 1): S219–S223
Todkar SS, Chavan VV, Kulkarni AS (2010). Screening of Secondary Metabolites and Antibacterial Activity of *Acacia concinna*. Res. J. Microbiol. 5: 974-979.
Trinder P (1996). Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann. Clin. Biochem., 6: 24-27.