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The ethanol extracts of *Rivina humilis*exhibits i*nvitro*antidiabetic properties by inhibiting α-glucosidaseandα-amylaseenzymes

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Running Title: α-Glucosidase and **α**-Amylase inhibitor activity of *Rivina humilis*

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ABSTRACT

Objective: The current investigation was carriedout to investigate *in vitro* anti-diabetic potentials of ethanol extract of ariel parts of *Rivinanhumilis*against by α-glucosidase and α-amylase inhibitory activities.

Materials &Methods: The ariel parts of plant *Rivinahumilis* were collected and authenticated. dried and powdered. The powdered drug was defatted with petroleum ether and subjected to ethanol extraction. The ethanol extract of *Rivinanhumilis* was subjected to preliminary phytochemical investigation. The *in vitro*α-glucosidase inhibitory and α-amylase inhibitory properties were determined for the ethanol extract and concentrations of extract required to inhibit absorbance (IC_{50} values) of EERH were determined.

Results: The bothstudies conducted to evaluate α-glucosidase and α-amylase inhibitoryactivity have exhibited significant IC_{50} values indicating the ability of ethanol extract of *Rivina humilis* to inhibit carbohydrate digestive enzymes and to prevent postprandial blood glucose.

Conclusion: The results of the presents investigation recommends that ethanol extract of *Rivina humilis*possess significant α-glucosidase and α-amylase inhibitoryactivity.

Key words: Antidiabetic activity, *Rivina humilis*,α-glucosidase and α-amylase and IC_{50}values.

I. INTRDUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder which results from the lack of insulin secretion in the body and leads to disturbances in carbohydrate, protein and lipid metabolism. Besides typical symptoms like hyperglycemia, weight loss, polyurea and polydypsia, Diabetes mellitus has several other symptoms thatincludes hyperlipidemia, which are involved in the development of micro-vascular and macrovascular complications of diabetic patient and may leads to death [1,2]. Type II diabetes mellitus, also known as non-insulin dependent diabetes mellitus, which develops in middle or later life and affects 2–6% of adults in most Western societies.[3]i Diabetes mellitus (DM) is the most common health problem of the world in the current century. Nowadays more than 366 million people suffer from DM and according to World Health Organization estimates, 552 million are expected to be affected by diabetes by 2030.[4]Treatment of hyperglycemia in diabetes involves diet control, exercise and the use of hypoglycemic drugs (oral). Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies, due to the side effects associated with these therapeutic agents. Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are widely prescribed even when their biologically active compounds are unknown [3].

The use of pharmacological and chemical agents currently available for the treatment of type II diabetes include sulfonylurea, biguanide, thiazolidinedione and α glycosidase inhibitors are known to possess several undesirable side effects and fail to significantly alter the course of diabetic complications. At present, insulin is the choice of drug for the treatment of insulin dependent diabetes mellitus (Type I IDDM) where as other synthetic

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drug like sulfonylureas and insulin sensitizers are the effective drugs for curing non insulin dependent diabetes mellitus (Type II-NIDDM). But these drugs possess very serious and potential adverse effects like cardiotoxicity, nephrotoxicity and etc [4]. Hence irrespective of tremendous advancements in the medical field there is no truly satisfactory drug available for the treatment of diabetes mellitus. Hence always there is scope to develop drugs from plant origin which already effectively used in Indian traditional system like Ayurveda for treatment of diabetes mellitus and WHO always encourage the research activities from natural sources to prevent the high prevalence of diabetes as well as its long term complications.Since ancient times herbal remedies have been used for the treatment of diabetes mellitus. About 90% of the world population in rural areas of developing countries relies solely on traditional medicines for their primary health care [4,5].

Plants have a special place in the treatment of cancer. It is estimated that plant derived compounds one or the other way constitute more than 50% of anticancer agents[6,7]. The *Rivina humilis* also known as belongs to the family anacrdiaceae native of India, Nepal, Myanmar and Srilanka. *Rivina humilis*is the plant having very good medicinal value. In old ancient medicinal system, it was used for the treatment of diabetes and other diseases. However, there is paucity ofscientific data to support this activity and hence the present study was designed to evaluate the in vitro antidiabetic activity of ethanol extract by α-Glucosidase and α-amylase inhibitory activity of *Rivina humilis*[6,7].

II. MATERIALS &METHODOS

Plant material

The whole*Rivina humilis*plant was discovered in the Chittor Forest, confirmed by Dr. MadhavaChetty, Assistant Professor of Botany, and stored at the institute herbarium library. For further use, the verified leaves were separated from other plant components, cleaned, rinsed, and dried.

Preparation of the ethanol extract

The plant material was collected and dried under shade. The dried leaves are then powdered and the coarse powder will be defatted with petroleum ether. The defatted powdered drug will be subjected to ethanol extraction in soxhlet apparatus for 48 hours and the marc left over will be subjected to aqueous extraction using chloroform water [8].

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the ethanol (EEERH) of *Rhusmysorenis*was conducted as per procedure prescribed by Khandelwal [9].

Evaluation of in vitro antidiabetic activity of extract of *Rivina humilis*

α-Glucosidase inhibitory assay

The test was executed to explore *in vitro* inhibitory potentials of TPME on carbohydrate digestive enzyme α-glucosidase for sucrose and maltase in GIT. In spite of α-glucosidase enzyme separated from yeast is considerably used for the evaluation of α-Glucosidase inhibitor drugs, the results may not always in accordance with those obtained from mammal enzymes.

Hence in present research study small intestine homogenate of albino mice was used as solution of alpha-glucosidase enzyme since it postulated that it would better reflect the *in vivo* physiological state. The glucosidase and amylase inhibitory activity of EERH was measured by slightly modifying the methods used in previous research studies. The segment of the small intestine of experimental mouse duodenum and cecum was cut and removed after 20 hours of fasting. The part of intestine collected was rinsed using ice-cold normal saline solution, and subjected to homogenization with 12 mL of maleate buffer (100 mM, pH 6). The homogenate substance acquired was utilized as α-glucosidase solution for further investigation. The reaction mixture of assay composed of 100 mM maleate buffer (pH 6), 2% (w/v) of sucrose and maltose substrate solution (100 ml), and ethanol extract of *Rivina humilis* (20-640 µg/mL). After the preincubation for5 minutes at 37^{O}Creactionmixture, the reaction was started by adding raw α-glucosidase enzyme solution (1 ml), which is again incubated for 10 minutes at 37^{O}C. The quantity of glucose generated in present reaction was estimated by a glucose assessment kits (Span Diagnostic Ltd., Mumbai, India). The amount of glucose released by the positive control (GCP), glucose generation blank value (GCB) and quantity of glucose produced by the addition of EERH (GCT) were noted [10,11].The rate of carbohydrate degradation was assessed as a percentage ratio to the quantity of glucose generated when the carbohydrate was entirely degraded. The rate of inhition was determined by the following formula:

$$\text{Inhibition rate (\%)} = \frac{\text{GCP- GCT-GCB}}{\text{GCP}} \text{ X100}$$

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α-Amylase inhibitory assay

The assay samples of methanol extract of *Rivina humilis* at serial concentrations (6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL, 100 mg/mL, 200 mg/mL) and reference standard nojirimycin (6.25-200 µg/mL)] of 500 ml were added to 500 ml of 0.02 M sodium phosphate buffer (at pH 6.9 with 0.006 M sodium chloride) containing 0.5 mg/mL porcine pancreatic enzyme alpha-amylase solution and were kept for incubation for 10 minutes at temperature 25^{O}C. After the pre-incubation, 500 ml of solution of 1% starch in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was incorporated to every assay at predetermined time intervals. The assay mixtures were then incubated for 10 minutes at 25^{O}C. The reaction of assay was concluded by incorporating 1 mL of 3,5- dinitro-salicylic acid color reagent. The all test tubes were kept for incubation in a water bath at boiling condition for 5 minutes and cooled to normal room temperature under tap water. The assay was then diluted by incprporating10 mL of distilled water and absorbance was recorded at 540 nm [10,11].

$$\% \text{ inhibition} = \frac{\text{Abs(Control)(540) - Abs (Extract)(540)}}{\text{Abs (Control)(540)}}$$

III. RESULTS

Evaluation of in vitro anti-diabetic activity

α-Glucosidase inhibitory activities

An *in vitro*α-glucosidase enzymes inhibitory test was performed to estimate the inhibitory potentials ofmethanol extract of *Rivina humilis*. The half of maximal concentration requiresinhibitingsucrose and maltase enzymes (IC_{50}) for EERH were 399.73µg/mL and 289.36µg/mL and for standard acarbose103.43µg/mL and 96.93µg/mL respectively. This indicatesthat EERH exhibited potent propertywhich depends on dose and is thus considered to be an powerful α-glucosidase inhibitory drug[Table.1 and Figure 1 and 2].

α-Amylase inhibitory activities

Toassess the inhibitory activity of methanol extract of *Rivina humilis on* postprandial glucose rise, an in vitro α-Amylaseinhibition test was performed. In this study, EERH have shown strong inhibitory action against α-amylase with IC_{50} of 25.81µg/mL and 101.92µg/mL which was comparable with reference standard [Table 1 and Figure.3].

Figure 1: Effect of EERH on α-Glucosidase (Sucrase) and amylase inhibitory activity.

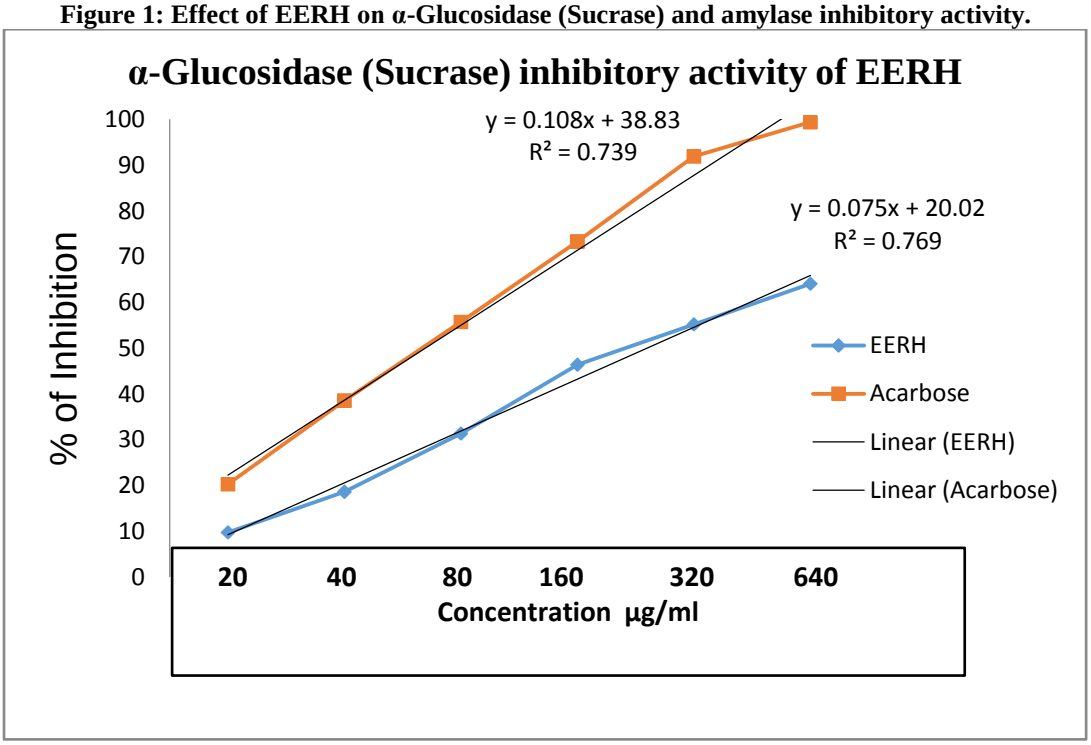

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Figure 2: Effect of EERH on α-Glucosidase (maltase) inhibitory activity

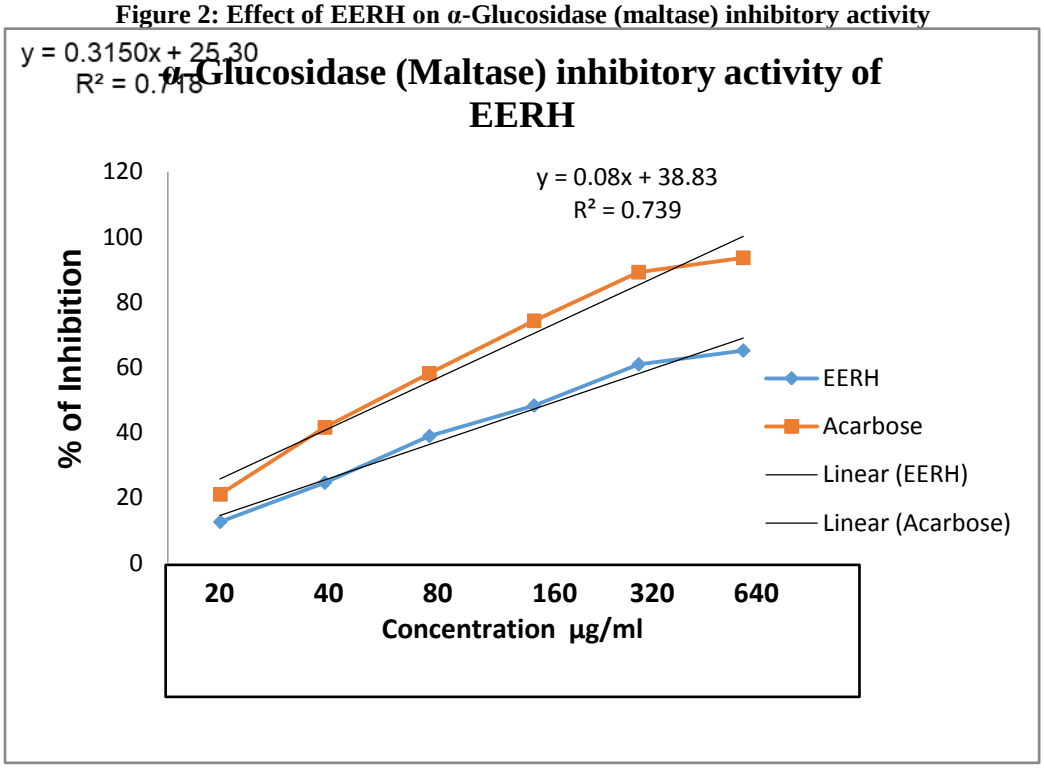

Figure 3: Effect of EERH on amylase inhibitory activity

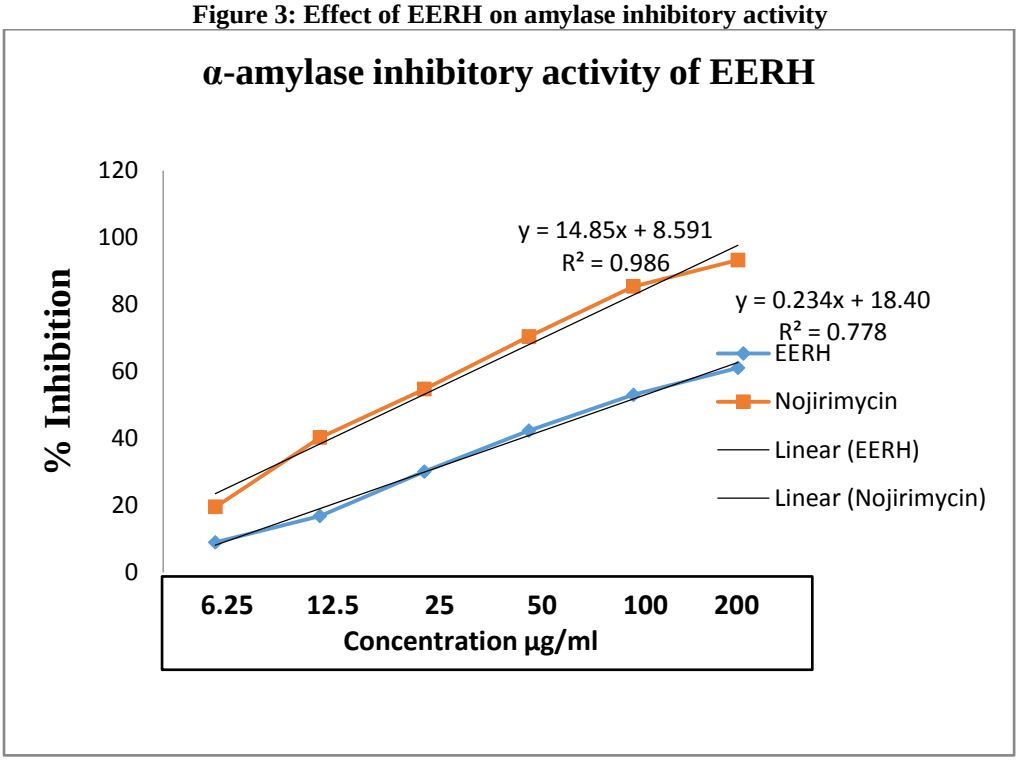

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Table 1: IC50 values of EERH for glucosidase and amylase inhibitory activities

Sl.No	Name of Drug	IC50 Valuesµg/mL		
		Sucrase	Maltase	Amylase
1.	**EERH**	399.73	289.36	101.92
2.	**Acrabose**	103.43	96.93	-
3.	**Nojirimycin**	-	-	25.81

IV. DISCUSSION

The novel therapeutic approach for management of diabetes mellitus is inactivation of carbohydrate hydrolyzing enzymes like α-amylase and α-glucosidase enzyme to counter the absorption of glucose from GIT and thereby to reduce the postprandial hyperglycemia and it problems [12,13].The α-glucosidase enzyme inhibition by EERH was examined by performing the α-glucosidase inactivity potential with 4-Nitrophenyl-b-D-glucopyranosiduronic acid (pNPG) as the reaction precursor using small intestine of as a source of α-glucosidases, sucrase and maltase [14,15]. In the present investigation the EERH have exhibited significant α-glucosidase and α-amylase inhibitory properties indicates its usefulness to reduce postprandial glucose but still it is not clearly understood whether the inactivation of α-amylase enzyme and α-glucosidase enzyme by EERH is due to competitive or noncompetitive inhibition mechanisms. However, the rate of inactivation for α-glucosidase enzyme was near to that of acarbose a reference standard drug used in study, but the inactivation rate for α-amylase was little than that of standard drug. This shows that EERH is a potent inhibitor of α-glucosidase with less potent inhibitory property versus α-amylase. The α-glucosidase along with α-amylase enzyme inhibitory properties of EERH can regarded to be a productive approach for the prevention of diabetes mellitus by declining the uptake of glucose into blood. Significant post meal hyperglycemia very commonly experienced by patients with diabetes could be controlled if the rate of uptake glucose from the GIT into the blood circulation could be declined by inactivating hydrolysis of carbohydrate [16].

Skeletal muscle comprises about 30-40% of the total quantity of body and hence it can be one of the most major target tissues for the activity of insulin which enhances the utilization of glucose at the peripheral level. It is a well understood that insulin and anti-diabetic drugs stimulate glucose utilization by peripheral cells and tissues. Other major finding of the present study is that EERH have significant action similar to insulin as witnessed by the stimulation of glucose utilization from the rat's hemidiaphragm, which constitutes muscle tissue that are essential tissues of insulin regulated glucose discharge. The EERHconsiderably enhanced the uptake of glucose by isolated rats muscle hemidiaphragm and is observed to be less potent than insulin. It seems that EERH has action on peripheral tissues and results of normal group of glucose utilization by rat peripheral tissue corresponds with those of earlier findings [17].

In spite there is no clear specific mechanism of alloxan responsible for pancreatic damage understood, investigations propose that the alloxan destroys pancreatic β cells due to its free radical nature which followed by absolute insulin deficiency and diabetes mellitus [18,19]. The previous researches conducted have suggests that antioxidant activity can be one of the possible mechanism of action for antidiabetic activity that protects pancreatic cells against oxidative damage. Hence further study can be performed to explore antioxidant activity of EERH to determine its ability to reduce reducing insulin resistance which is also important mechanism required for antidiabetic activity [20,21].In the current *in vivo* assay the ethanol extract had been effective to stimulate insulin secretion and to regulate the normal glucose level in the therapeutic groups. The study should be conducted to determine the antioxidant properties of EERH which is possible mechanism of action in the present study that can defend pancreatic cells against alloxan mediated damage and normalize the insulin release. In *in vitro* findings the EERH exhibited its potency to counter insulin resistance by increasing the utilization of glucose by peripheral tissues and extract also exhibited its potentials in inhibiting GIT digestive enzymes to prevent complications of post-prandial hyperglycemia.

V. CONCLUSION

The results of the presents investigation recommend that ethanol extract of *Rivina humilis* possess significant α-glucosidase and α-amylase inhibitory activity. But further examination is necessary to isolate and estimate the specific components present in methanol extract of *Rivina*

*humilis*that may be responsible for these beneficial properties to improvethe health conditions connected with diabetes mellitus.

CONFLICT OF INTEREST

All authors are hereby declaring that there is no conflict of interest with respect to manuscript.

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REFERENCES

[1]. Linda S. BRS physiology. 4th Ed. Constanzo; Lippincott publishers, 2006:255-6.
[2]. Tortara GJ & B Derrickson. Principles of anatomy and physiology; The digestive system. 13 ed. USA;2011.:967-1023.
[3]. Waugh A, Grant A, Ross and Wilson. Anatomy and physiology; the digestive system. 9th ed. USA;Elsevier:2001: 281-338
[4]. Brunicardi FC. Pollock Schwartz's Principles of surgery. 9th Ed. USA; McGrawHill:2009: 1265-73.
[5]. Stephen JP. The Exocrine Pancreas. Colloquium series on Integrated Systems Physiology: From Molecule to Function; Shreveport2011:3(1):1-64.
[6]. Muhammad A, Annam Z, Khalid MK, Shahnaz P, Shazia Shah and Aneela K. Rivina humilisL: A Potential Antimicrobial and Antioxidant Source. J ChemSoc Pak 2013;35(5):1384-98.
[7]. Sushama PR, Mehtab Y, Prabhakar G, Hajera S and Kaneez F. A Report on Rivina humilisL. an Invasive Alien Weed from Telangana State, India. Int J Crea& Res Thoughts 2021;9(11):175-80.
[8]. Kokate CK. Practical Pharmacognosy. VallabhPrakashan New Delhi: 1994;4:110-1.
[9]. Khandelwal KR, Practical Pharmacognosy-techniques and experiments. Pune; NiraliPrakashan; 2000.
[10]. Dahlqvist A. Method for assay of intestinal disaccharidases. Anal Biochem. 1964;7:18-25.
[11]. Kwon GJ, Choi DS and Wang MH. Biological activities of hot water extracts from Euonymus alatus leaf. J Korean Food Sci Technol. 2007;39:569-74.
[12]. Puls W, Keup U, Krause HP, Thomas G, Hoffmeister F. Glucosidase inhibition. A new approach to the treatment of diabetes, obesity and hyperlipoproteinaemia. Naturwissenschaften.1977;64:536-7.
[13]. Toeller M. a-Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. Eur J Clin Invest. 1994;24(Suppl. 3):31-5.
[14]. Chiba S. Molecular mechanism in a-glucosidase and glucoamylase. BiosciBiotechnolBiochem. 1997;61:1233-9.
[15]. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and a-glucosidase activities by tea polyphenols. BiosciBiotechnolBiochem. 1993;57:123-4.
[16]. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes. 1981;30:1000-7.
[17]. Melander A. Glucose uptake by hemidiaphragm. Diabet Med. 1996;13:143.
[18]. Cohen G, Heikkila KE. Generation of hydrogen peroxide, superoxide radical by 6-hydroxy dopamine, dialuric acid and related agents. J Biol and Chem 1974; 49:2447-52.
[19]. Okamoto H. Molecular basis of experimental diabetes: Degeneration, oncogenesis and regeneration of pancreatic β-cells. Bio Essays 1995; 2:15-21.
[20]. Takasu N, Asawa T, Komiya I, Nagaswa Y, Yamada T. Alloxan-induced DNA strand breaks in pancreatic islets:Evidence for H_2O_2 as an intermediate. J Biol and Chem 1991;266:2112-4.
[21]. Mishra MR, Mishra A, Pradhan DK, Panda AK. Antidiabetic and Antioxidant Activity of *Scopariadulcis* Linn. Indian J Pharm Sci 2013;75(5):610-14.