IJPSR (2013), Vol. 4, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 November, 2012; received in revised form, 05 January, 2013; accepted, 27 February, 2013

ANTI-DIABETIC ACTIVITY OF *RIBES NIGRUM* FRUIT EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

G. Sivalingam* and N. Sriram

Smt. Sarojini Ramulamma College of Pharmacy, Seshadrinagar, Mahabubnagar, Andhra Pradesh, India

Keywords:

Ribes nigrum, Antidiabetic activity, Alloxan-induced diabetic

Correspondence to Author:

N. Sriram (M. Pharma, Ph. D)

Associate Professor, Smt.Sarojini Ramulamma College of Pharmacy, Seshadrinagar, Mahabubnagar, Andhra Pradesh, India

E- mail: sriramphar ma@gmail.com

ABSTRACT:

Different doses of Ethanolic fraction of *Ribes nigrum* fruit were evaluated for hypoglycemic activity in Alloxan induced diabetic rats. The oral administration of Ethanolic extract at a dosage of 100mg/kg and 500mg/kg body weight exhibited significantly reduced the serum glucose level in both acute (1, 3, 5 h) and sub-acute (1, 3, 5, 7 days) treatments. Perform the Histopathological study of pancreas.

INTRODUCTION: Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia, glycosuria, polyurea and polydipsia induced by insulin deficiency [1] and insulin resistance [2]. Recent estimates indicate that there were 171 million people in world with Diabetes in year 2000 and this may be projected to increase to 366 millions by 2030 [2].

Diabetes mellitus is treated by using Oral hypoglycemic agents such as Sulphonyl Ureas. Biguanides, Meglitinides and Alpha glucosidase inhibitors. Traditional medicines like herbal drugs in primary form or their extracts have been used by many diabetic patients as they are assumed to be non–toxic in nature

About plant: *Ribes nigrum* is a common name of Blackcurrant it belongs to Grossulariace family. It is geographically distributed in northern India, northern Asia, and central Europe It is a small shrub, growing to 1–2 m tall. The fruit is an edible berry 1 cm diameter, very dark purple in colour, almost black, with a glossy skin and a persistent calyx at the apex, and containing several seeds dense in nutrients. An established bush can produce up to 5 kilograms of berries during summer. [3]

MATERIAL AND METHODS:

Collection of plant material: The fruit of *Ribes nigrum* were collected and authentication was done by botanist Dr. Madhava chetty, Assistant Professor, Department of Botany in S.V. University, Titupathi. The voucher no. 1006

Preparation of the extracts: The plant materials were dried in the shade and powdered by a mechanical grinder. The powder of *Ribes nigrum* fruits was extracted with 90% Ethanol.powedered material and solvent was used at 1:4 ratio, at 65-70^{0}C by using a Soxhlet extractor for 12 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered using whattman filter paper (No 1) and then concentrated in a vacuum and dried at 45˚C for ethanol elimination. The extracts were kept in a sterile bottle under refrigeration conditions of about 2-8˚C [4, 5].

The percentage yield (20%) was calculated. The extracts were used for further studies and determination of activity.

Animals: Wister albino rats (150–220 g) of male were purchased from Sainath Agencies, 1-6-197/45/D, Bapujinagar, Musheerabad, (Reg. No. 282/99/CPCSEA), Hyderabad 500048, India and Maintained under standard environmental laboratory conditions and fed with laboratory diet and water *ad libitum*. All the protocols were performed in accordance with the Institutional Animal Ethical committee (IAEC) as per the directions of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) All the pharmacological studies are carried out at Smt. Sarojini Ramulamma college of Pharmacy. (Reg. No: 51/01/c/cpcsea/2012/16).

Limit test: Albino Wistar Rats are taken for the study of limit test. Three animals treated with 2000mg/kg as a single dose via oral route as per the limit test of OECD guide lines 423 and. The rats were observed continuously for 24 h for behavioral, neurological, and autonomic profiles and, after a period of 24 and 72 h, for any lethality, morbidity state or death. The animal not showed any signs of toxicity and behavioral changes after 24 hrs and 72 hrs [6].

Hypoglycemic activity:

Induction of diabetes: Alloxan monohydrate was first weighed individually for each animal according to its weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 100 mg/kg b. wt. intraperitonially. After 1 hr of Alloxan administration, the animals were given feed *ad labium* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase.

The animals were kept under observation and after 48 hr, blood glucose was measured. One group served as a control which received vehicle alone. The diabetic rats (glucose level > 150 mg/dl) were separated and divided into four different groups for experimental study [7].

Experimental design:

Acute treatment and sub acute treatment: Normal rats are kept into group, diabetic induced rat are grouped into 2, 3, 4, 5. Each group contains six rats.

1. Group I: Normal control (saline),
2. Group II: Diabetic control (saline),
3. Group III: Standard (Glibenclamide 10mg/kg)
4. Group IV: Test-dose (100mg/kg of *Ribes nigrum* fruit Extract)
5. Group V: Test-dose (500 mg/kg of *Ribes nigrum* fruit Extract)

Drugs are administered via oral route, Treatment continued for seven days [7].

Acute study (single day study): Blood samples were collected from rat tale vain and serum glucose levels were estimated at 0, 1, 3 and 5 h after the extract administration.

Sub acute stud (seven day study): Blood samples were collected from rat tale vain and serum glucose levels were estimated at 1, 3, 5, and 7 days Blood glucose levels were determined by god-pod method.

Histopathological study of Pancreas: Rats are sacrifice by using euthanasia. Pancreases are collected in 10% formalin solution. The section cuttings of 5μ thickness were is stained with haematoxylin and eosin. The section was observed in the Photomicrograph [8].

Statistical analysis: All parameters were analyzed statistically by using Graph pad Prism version 5.0.Rats was analyzed by one way ANOVA followed by "Dennett's Test". All the values of the experimental results were expressed as mean±sem. Differences were considered significant at $p < 0.05$ [9].

RESULTS:

TABLE 1: EFFECT OF *RIBES NIGRUM* FRUIT EXTRACT ON SERUM GLUCOSE LEVEL FOR ACUTE STUDY (SINGLE DAY STUDY)

Treatment	Blood glucose level (mg/dl)			
	0 h	1 h	3 h	5 h
Normal control	88.67±2.305	89.17±2.242	89.33±2.108	89.17±1.990
Diabetic control	181.5±4.233	183.5±4.280	187.7±4.440	190.3±4.201
Standard	178.3±4.248	171.0±4.250	156.2±4.534**	148.3±4.271***
Extract 100mg/kg	180.7±4.279	177.2±4.549	171.7±4.828	168.5±4.537
Extract 500mg/kg	176.7±3.703	171.3±3.343	157.8±3.458**	148.5±3.433***

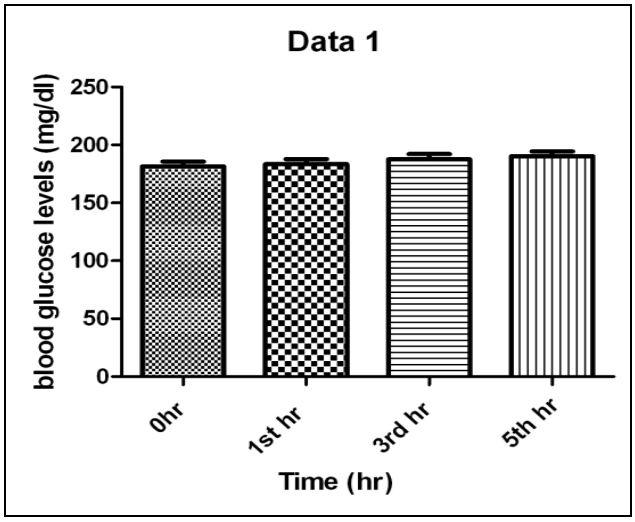
Values are mean±SEM; N=6. *P value < 0.01, **p < 0.001, ***p < 0.0001vs. Diabetic control.

TABLE 2: EFFECT OF *RIBES NIGRUM* FRUIT EXTRACT ON BLOOD GLUCOSE LEVEL FOR SUB ACUTE STUDY (MULTI DAY STUDY)

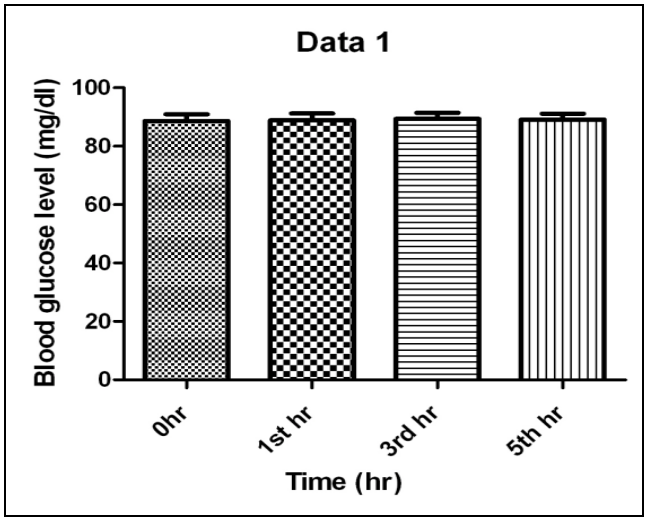
Treatment	Blood glucose level (mg/dl)				
	0 day	1 day	3 day	5 day	7 day
Normal control	88.67±2.305	89.34±2.073	88.50±2.141	88.50±2.029	88.50±1.928
Diabetic control	181.5±4.233	187.2±4.296	196.5±4.161	202.5±4.145	208.5±4.039
Standard	178.3±4.248	158.4±4.373**	149.8±4.438***	132.0±4.553***	111.3±4.558***
Extract 100mg/kg	180.7±4.279	172.4±4.636	168.0± 4.328	164.8± 4.061*	160.7± 3.955**
Extract 500mg/kg	176.7±3.703	159.2±3.402**	149.0±3.406***	132.7±3.499***	117.3±4.372***

Values are mean±SEM; N=6. *P value < 0.01, **p < 0.001, ***p < 0.0001vs. Diabetic control.

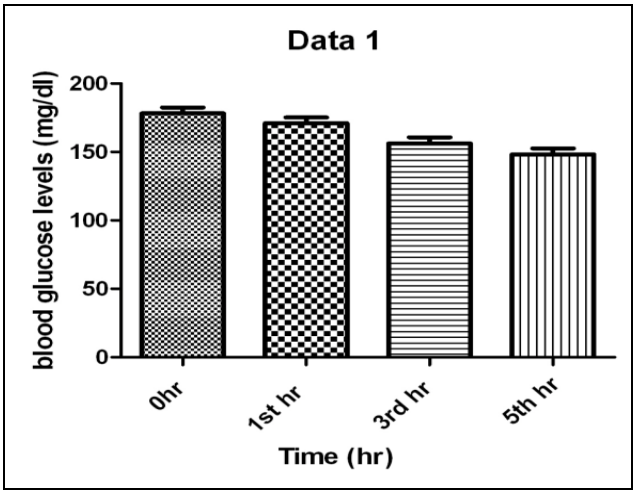
Graphical representation of serum glucose levels in the acute study:



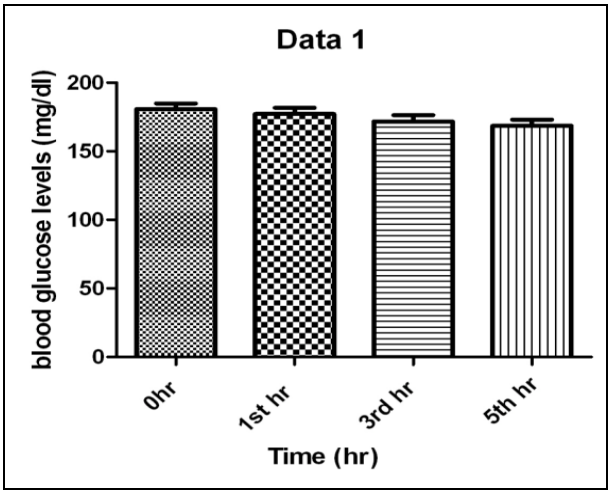
(A) Diabetic control



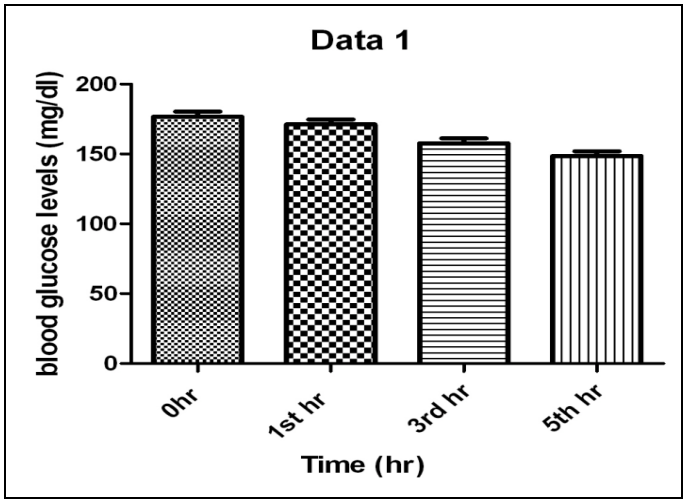
(B) Normal control



(C) Standard



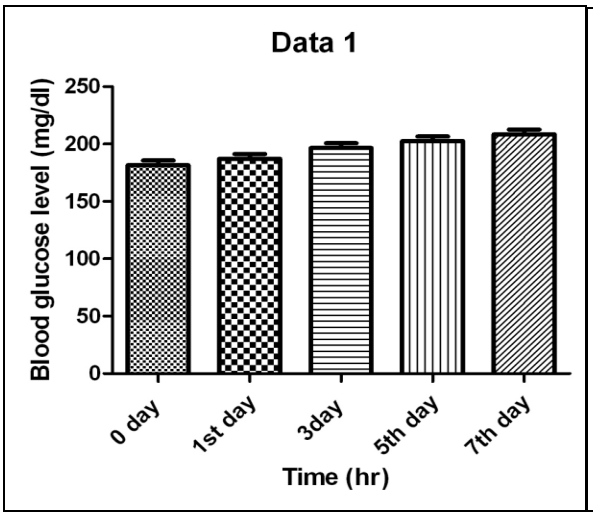
(D) Extract 100mg/kg



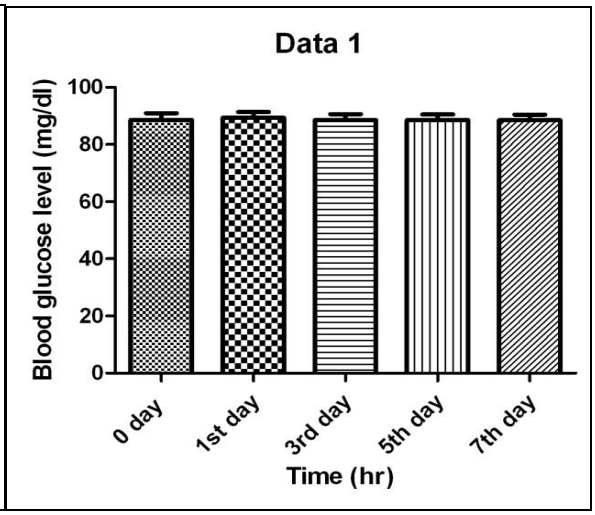
(E) Extract 500mg/kg

FIG. 7.1. GRAPHICAL REPRESENTATION OF BLOOD GLUCOSE LEVELS IN THE ACUTE STUDY (A) DIABETIC CONTROL, (B) NORMAL CONTROL, (C) STANDARD, (D) EXTRACT 100mg/kg AND (E) EXTRACT 500mg/kg

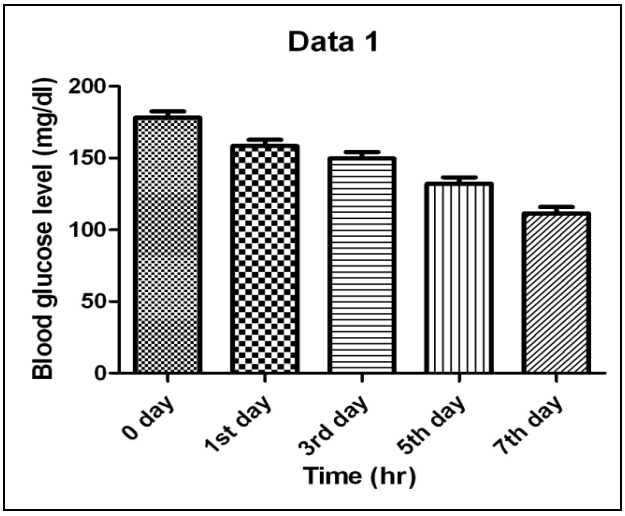
Graphical representation of serum glucose levels in the sub acute study:



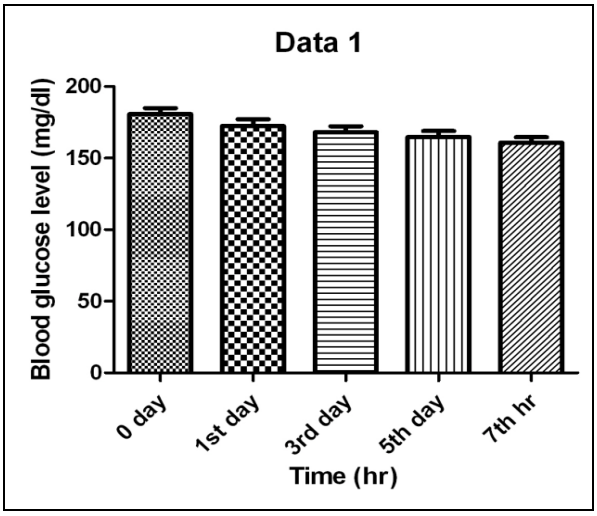
(A) Diabetic control



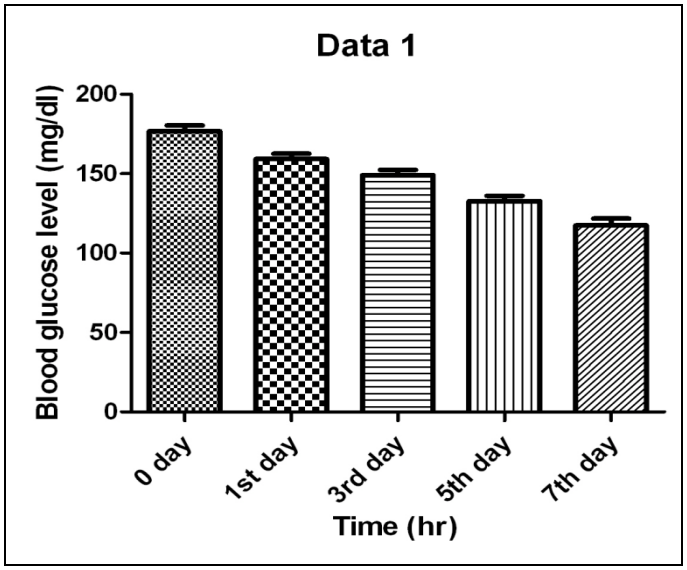
(B) Normal control



(C) Standard



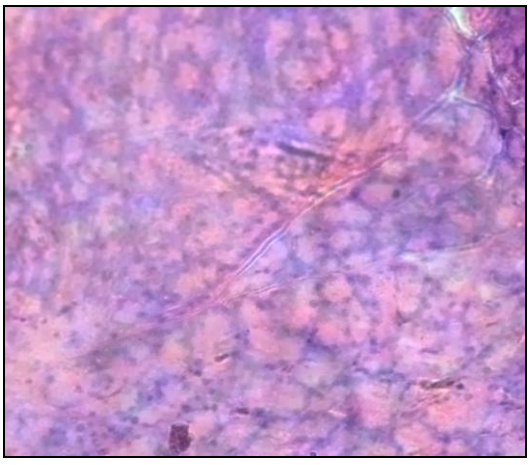
(D) Extract 100mg/kg



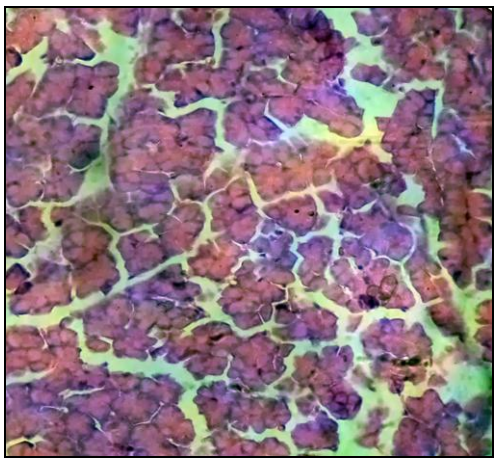
(E) Extract 500mg/kg

FIG. 7.2: (A) DIABETIC CONTROL, (B) NORMAL CONTROL, (C) STANDARD (D) EXTRACT 100mg/kg (E) EXTRACT 500mg/kg

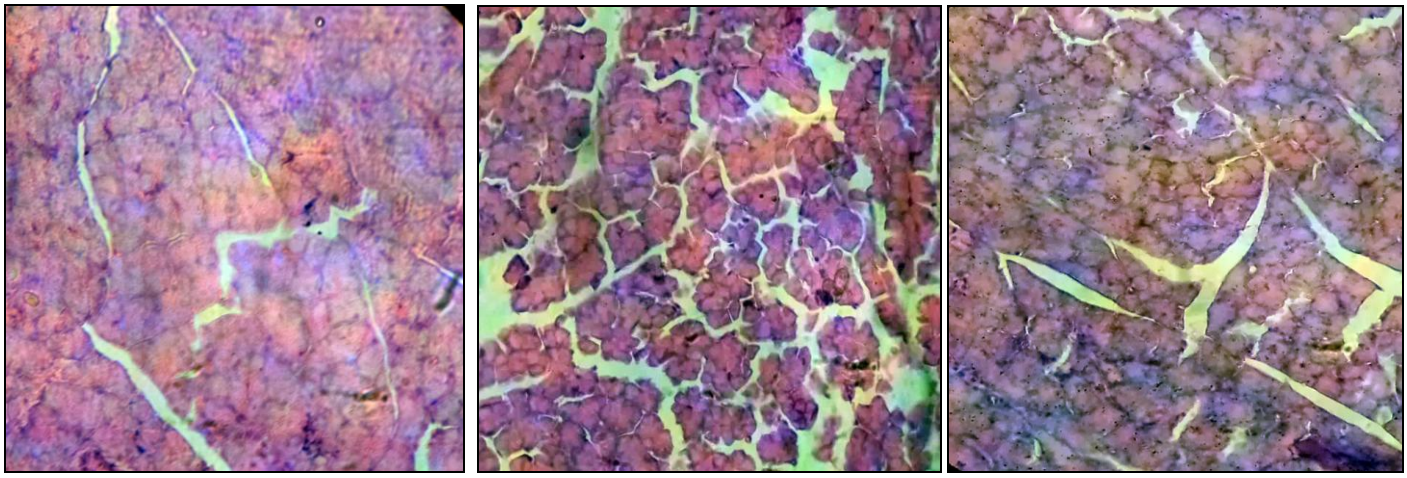
Histopathological study: The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution. Sections of 5µ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination.



NORMAL CONTROL



DIABETIC CONTROL



STANDARD **TEST 100MG/KG** **TEST 500MG/KG**

DISCUSSION:

- In acute study, the *Ribes nigrum* fruit extract at the dose of 500 mg/kg shows very significant Antidiabetic activity at 3rd hour, the dose of 100 mg/kg did not show any significant antidiabetic activity.
- In sub acute study the *Ribes nigrum* fruit extract at the dose of 500 mg/kg shows very significant activity from the first day to seventh day, the dose of 100mg/kg shows significant from 5th day to 7th day.
- Histopathological study shows that 500 mg/kg extract have shows high level regeneration of beta cells as compared to 100 mg

REFERENCES:

1. World Health Organisation Department of Noncommunicable Disease Surveillance (1999). "Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications"(PDF).
2. Marcia da silva pinto, Evaluation of red currents (*Ribes rubrum* L.), black current (*Ribes nigrum* L.), Red and Green Gooseberries (*Ribes uva-crispa*)for potential management of type 2 diabetis and hypertention using *in-vitro* models, Journal of Food Biochemistry, vol 34, issue 3, 639-660, june 2010.
3. En.wikipedia.org/wiki/black currant.
4. En.wikipedia/wiki/ethanol.
5. En.wikipedia.org/wiki/Soxhlet_extractor.
6. Acute Oral Toxicity – Acute Toxic Class Method, OECD Guideline For Testing Of Chemicals, 423, 17th December 2001.
7. S. Venkatesh , J. Thilagavathi, D. Shyam sundar, Anti-diabetic activity of flowers of Hibiscus rosasinensis, Fitoterapia 79 (2008) 79–81.
8. Mohammed Fazil Ahmed, Syed Mohammed Kazim, Syed Safiullah Ghori, Syeda Sughra Mehjabeen, Shaik Rasheed Ahmed, Shaik Mehboob Ali, and Mohammed Ibrahim, Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats, International Journal of Endocrinology, Volume 2010.
9. V. Gomathi, B. Jayakar, R. Kothai, G. Ramakrishnan, Antidiabetic activity of leaves of *Spinacia oleracea* Linn. in Alloxaninduced diabetic rats, *J. Chem. Pharm. Res., 2010, 2(4): 266-274.*
10. Mohammed Fazil Ahmedet al, Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats, International Journal of Endocrinology, Volume 2010, Article ID 841090, 6 pages, doi:10.1155/2010/841090.

How to cite this article:
Sivalingam G and Sriram N: Anti-diabetic activity of *Ribes nigrum* fruit extract in Alloxan induced diabetic rats, 2011. *Int J Pharm Sci Res* 2013; 4(3); 1196-1201.