Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 6 (2)

Hypoglycaemic and Antilipaedemic Effects of Aqueous Extract of Cinnamon and Insulin in Streptozotocin-Induced Diabetic Rats: A Comparative Study.

Odiase DE and Om'Iniabohs FAE

Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

Corresponding Author: Odiase DE

E-mail: odiase2002@yahoo.com; +234806710618

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder which is characterized by abnormalities in carbohydrate, protein and lipid metabolism. The present study was undertaken to evaluate the comparative effects of aqueous cinnamon extract and insulin on the liver of streptozotocin-induced diabetes in Wistar rats. Twenty-four adult wistar rats weighing between 220g and 300g were used and randomly selected into four groups of six rats per group. Group A served as the normal (non-diabetic) control group that recieved feeds and water *ad'libitum* throughout the period of the experiment. Groups B, C, and D were induced with diabetes by intraperitoneal injection of 50mg per kilogram of body weight of streptozotocin. Group B was treated with STZ only, group C was further treated with intramuscular injection of 3 I.U of insulin (humilin R) after STZ treatment, while group D was further treated with oral administration of 300mg/kg/day of crude aqueous extract of Cinnamon after STZ for 21 days. The weights of the animals and their random blood glucose levels were measured. The animals were fasted for 12hours before they were sacrificed on day 22nd. At the end of the experiment, the animals were sacrificed and their blood collected for biochemical assay while the organ (liver) was harvested and processed for microscopy and stained using Haematoxylin and Eosin. The result revealed significant changes in weights, blood sugar levels and lipid profile of the animals in the various treatment groups. Histological changes were also seen in animals of the treated groups except for the groups A and D which features were essentially normal.

Keywords: Anti-diabetic, Diabetic mellitus (DM), Insulin and Streptozotocin (STZ).

INTRODUCTION

Diabetic mellitus (DM) is a chronic metabolic disorder affecting approximately 4% of the population worldwide, and expected to increase to about 5.4% in 2025[1]. It is characterized by abnormalities in carbohydrate, lipid and lipoprotein metabolism which not only lead to hyperglycemia but also cause many complications such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis[2]. Diabetes mellitus is considered to be at an epidemic level by the World Health Organisation[3].

The hyperglycemia caused due to decreased insulin production is called Type-1 diabetes (Insulin Dependent Diabetes mellitus – IDDM), and hyperglycemia due to insufficient insulin utilization is called Type-2 diabetes (Non-Insulin Dependent Diabetes mellitus – NIDDM), others include gestational diabetes and maturity onset diabetes of the young (MODY). About 90% of patients are NIDDM, with insulin resistance playing a key role in the development of the disease[4]. Insulin resistance decreased stimulation of muscle glycogen synthesis, defects in glycogen synthases and hexokinase activity[5].

However, despite evidence demonstrating both direct and indirect effects of insulin on the liver, it has been hypothesized that control of the liver is primarily indirect[6]. Insulin's indirect effects included reduction of glucagon secretion in the pancreatic islet and decreased protein catabolism in muscle (which further reduces gluconeogenic precursor availability)[7].

Plants have been used for the treatment of diabetes since 1550 BC[8]. A number of plant species and herbs have a long history of traditional use in treating elevated blood sugar levels[9].

According to the World Health Organization, more than 70% of the world's population must use traditional medicine to satisfy their principal health needs. A great number of medicinal plants used in the control of the diabetes mellitus have been reported[10]. There are various medicinal plants in the world, which are the potential sources of the drugs.

Cinnamon is one of the traditional folk herbs used in

Korea, China, India and Russia for diabetes mellitus[10,11]. Today cinnamon is widely used in Ayurvedic medicine (traditional India medicine) to treat diabetes in India[12]. Cinnamon is the bark of the Cinnamomi cassia[13], with its main constituents as Cinnamon aldehyde[14]; cinnamamic acid[15]; and methylhydroxychal cone polymer[16].

In addition, the aqueous Cinnamon extracts has been shown to potentiate the hypoglycemic effect of insulin [17]; a good anti-oxidant activity[18], as well as lowering triglyceride levels and serum cholesterol[19,20,21]. Therefore the present study was to compare the anti-diabetic effects of aqueous extract of Cinnamon and insulin in streptozotocin-induced diabetes mellitus in Wistar rats.

MATERIALS AND METHODS

Plant Material and Preparation of Extract

The bark of Cinnamomi cassia was purchased from the Vegetable Market at Aideyan Road, in the Government Reservation Area (G.R.A), Benin City, Nigeria. The plant bark was identified and authenticated by Sunny Nweke of the Herbarium Unit, Department of Plant Biology, University of Benin, Benin City, Nigeria. The quills of cinnamon were allowed to dry under shade and ground into powder form in a milling machine used in grinding plant samples. 1.428kg of the powdered material was packed into soxhlet apparatus and extracted using 1.6liter of distilled water. The extract obtained was concentrated using evaporation dish to yield 1.02kg of crude aqueous extract referred to as crude aqueous extract of Cinnamon.

Experimental Animal

Adult Wistar rats of both sexes with average weight of 275g were purchased, and maintained in standard animal cages from the Animal House Section of the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were left to acclimatize to laboratory conditions for two weeks and subsequently employed to testing for three weeks, during which time, they were fed with commercially formulated rat feed and water *ad libitum*. The animals were exposed to normal room temperature and lighting conditions and handled according to standard protocols for the use of laboratory animals [22].

Chemicals

Humilin (R) [intermediate acting insulin manufactured by Novo Nordisk, Denmark], Metformin tablets, manufactured by ZIM Laboratories LTD, Nagpur, India. 1g streptozotocin powder, produced by Sigma Aldrich UK. All other chemicals and reagents used were of analytical grade.

Induction of Experimental Diabetes

Diabetes was induced by a single intraperitoneal (i.p) injection of a freshly prepared STZ solution dissolved in 0.1M citrate buffer, p.H 4.5, at a dose of 50mg/kg body weight to overnight-fasted rats. At three days post-administration, rats with stabilized diabetes as indicated by a fasting blood glucose level of more than 250mg/dl, were selected for the study. Treatment was started on the fourth (4^{th}) day after STZ administration and continued for 21 days.

Experimental Design

In the present experiment, Diabetic rats were randomly assigned into four (4) groups (n=6) treated as follows: normal control rats received 5ml/kg normal saline (Group A); diabetic control rats received 5ml/kg normal saline (Group B); diabetic rats treated with 3IU/kg body weight/day of insulin (Group C) and diabetic rats treated with 300mg/kg body weight of crude aqueous extract of Cinnamon (Group D).

Body weight was monitored at 7 days intervals. After 21 days of treatment, the animals were euthanized. Blood was collected and liver samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible, and immediately stored at - 20 °C until analysis. An extra sample of liver was excised and fixed in 10% formalin solution for histopathology analysis.

Biochemical Analysis

During the experiment blood samples were collected from 12 hour-fasted rats by amputation of the tail tip under mild anesthesia**.** Plasma glucose was estimated by glucometer and glucometer strip (ACU-check advantage, Roche diagnostic, Germany; purchased from Pyrex Laboratories, Benin City). Triglycerides (T), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) were measured by using Span diagnostic reagent Kit. Very-low-density lipoprotein (VLDL) was calculated using formula High triglyceride (TG)/5. Low-density lipoprotein (LDL) concentration was estimated indirectly from the measured levels of TG, HDL, and TC using equation $LDL = TC - (VLDL + HDL)$.

Statistical Analysis

Data were evaluated with SPSS/10 software hypothesis testing methods that included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. *P* values of less than 0.05 were considered to show statistical significance. All these results were expressed as mean ± SEM for eight animals in each group.

RESULTS

Effects on Body Weight of Rats

The control group (group - A) gained weight over the three weeks of experimental period, with the mean body weight increasing by 23.8g after 3 weeks (Table 1). In contrast, the untreated diabetic group (group - B) lost a significant average weight of 42.4g after 3 weeks ($p<0.05$). Treatment with Insulin and crude aqueous extract of Cinnamon resulted in significant weight gain of 13.80g and 13.00g respectively to a level towards the

control group.

Effects of crude aqueous extract of Cinnamon on blood glucose level

The blood glucose level in diabetic group was significantly higher ($p<0.05$) than those of the control group (Table 2). On the other hand, administration of aqueous extract of Cinnamon for 21days was found to lower blood glucose level significantly in treated diabetic groups ($p<0.05$) when compared with those of the untreated diabetic group (group - B). The reference drug Insulin also lowers blood glucose level as compared to the diabetic control rats; however, the anti-hyperglycemic activity of the Cinnamon extract is less effective when compared to insulin

Serum Lipid Level

Serum total cholesterol (TC) and triglyceride (TG) levels were significantly elevated ($P < 0.001$) in STZ-induced diabetic group. Treatment of the diabetic animals with the extract at the above mentioned doses resulted in the values of these parameters tending towards the control level. Levels of TC and TG were significantly ($P < 0.05$) lowered towards the value of the control level as in crude aqueous extract of Cinnamon treated group than the value in insulin treated group (Table 3).

Lipidemic parameters like serum LDL levels were elevated significantly in STZ-induced diabetic group in comparison with the diabetic treated groups (insulin and Cinnamon treated). Treatment of the diabetic animals with aqueous extract of Cinnamon resulted in a significant reduction in serum LDL level than was recorded with the insulin treated group. However, insignificant variation was noted in the two levels.

Serum HDL level was decreased in STZ-induced diabetic group in contrast to the treated groups. Treatment of the diabetic rats with the composite (1:1) extract at the above mentioned dose revealed a significant ($P < 0.05$) recovery of this parameter towards the value of the control level. The composite insulin treatment resulted in significant recovery in the levels of these parameter also. An insignificant difference was noted in the level of HDL between the Cinnamon treated and insulin treated groups.

Serum Enzymes Profile (AST, ALT and ALP)

The study evaluated the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in all groups. A significant increase in AST was observed in STZ-induced diabetic group of Wistar rats. Moreover, treatment of diabetic rats with aqueous extract of cinnamon resulted in significant decreases in the levels of serum AST when compared to diabetic induced (untreated) rats and those treated with insulin. A significant level of reduction in serum-marker enzymes (ALT and ALP) were also noticed due to the ameliorating effect of insulin (Table 2).

Table 1: *Changes in body weight (wt.) in control, diabetic and diabetic rats treated with Insulin and crude Aqueous Extract of Cinnamon (AEC)*

GROUPS	A	B	C	D
Initial body wt. (g)	258.00±14.97	264.00±16.00	234.800±16.31	249.40±17.47
Final body wt. (g)	280.20±16.21	222.60±9.44	248.60±17.58	262.40±13.93
Change in body wt. (g)	+23.8	-42.4	+13.8	+13
Values in mean (±SD)	267.47±6.70	247.33±14.49	240.45±4.38	256.16±3.43

Values are expressed mean ±S.E.M
** P<0.05 as compared to diabetic induced rats*

Table 2: *Effects of aqueous extract of Cinnamon bark for 21 days on serum marker enzymes and serum sugar level on STZ-induced diabetic rats*

GROUPS	AST (U/l)	ALT (U/l)	ALP (KA unit)	Sugar (mg/dl)
A	11.00±1.29	6.75±.63	22.36±5.91	90.15±4.49
B	11.75±1.91	8.75±.75	50.96±14.91	394.40±110.55
C	14.00±3.82	6.00±.58	17.90±6.21	70.40±1.030
D	10.25±.63	7.50±1.23	18.42±7.3	120.20±3.023

Values are expressed mean ±S.E.M
** P<0.05 as compared to diabetic induced rats*

Table 3: *Effects of aqueous extract of Cinnamon bark on lipid profiles in control and experimental rats*

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
A	116.60±21.29	94.61±25.09	68.82±13.06	31.17±24.24
B	257.96±58.78	225.62±51.61	33.35±5.04	103.20±17.47
C	101.57±22.99	100.10±27.17	71.11±6.73	24.64±19.38
D	89.38±8.93	104.87±20.09	74.61±7.90	28.21±7.37

Values are expressed mean ±S.E.M
** P<0.05 as compared to diabetic induced rats*

Histological Studies

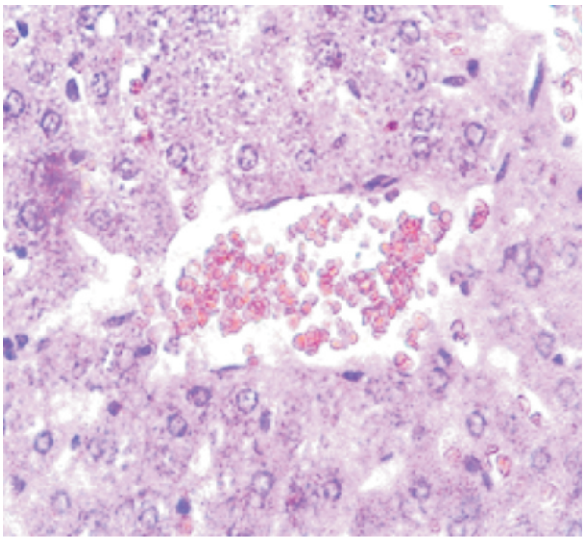

Figure 1: *(Control Rat Liver)*

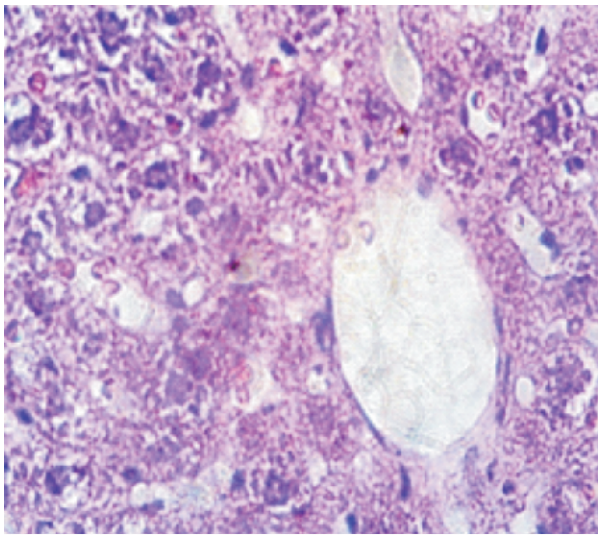

Figure 2: *(Diabetic Control Rat Liver)*

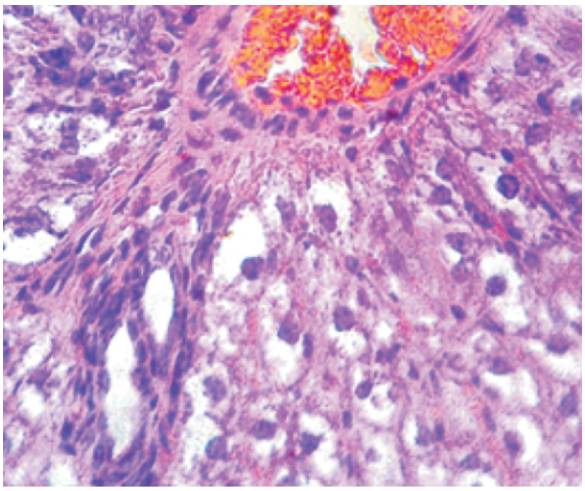

Figure3 :*(STZ-insulin treated Rat Liver)*

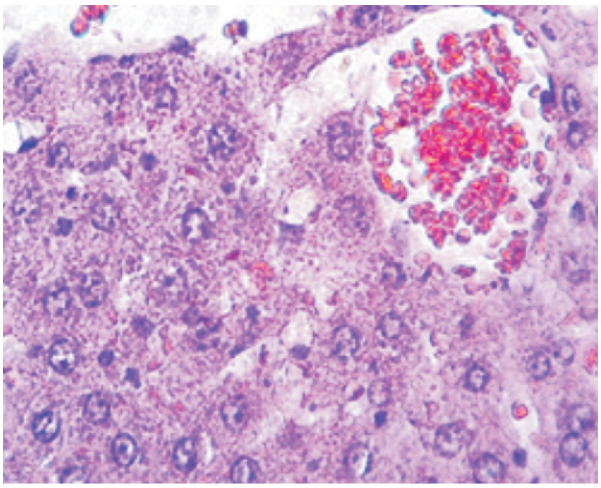

Figure4: *(STZ-cinnamon treated Rat Liver)*

Figure1–4: *illustrated the histological changes during investigations; they also provided essential evidence for the results of the biochemical analysis. In control rats (Fig. 1), liver sections showed normal hepatic cells radiating from the central vein, with well preserved cytoplasm and nucleus, whereas STZ-induced group of rats (Fig. 2) showed prominent halos around the nuclei which appeared distorted with fragmented nuclei and rampant vacuoles which were apparent signs of lipid infiltration. However, treatment with Insulin (Fig. 3) and Cinnamon (Fig. 4) improved the histological architecture when compared to (fig2). Haematoxylin and Eosin staining (X400)*

DISCUSSION

The morphological findings of weight gain affected both the experimental (groups treated with Insulin, aqueous extract of Cinnamon and STZ-treated only) and the control groups. The weights of the Wistar rats that were made diabetic by STZ administration and subjected to various treatment measures including aqueous extract of cinnamon and Insulin for 21 days were subjected to one way analysis of Variance (ANOVA). The Anova statistics indicated a highly significant difference ($p<0.01$), Table 1. The Duncan Multiple Range (DMR) test revealed that the weights of the diabetic rats (STZ-induced) that were treated with aqueous extract of Cinnamon and those treated with insulin were significantly higher than the weights of animals in the untreated group. The weights of the untreated diabetic group and the diabetic group treated with insulin were significantly different from each other. The increased weight-gain of diabetic Wistar rats treated with insulin could be attributed to good glycemic control of diabetes mellitus. Also the reduction in weights observed in the diabetic untreated group could be due to hyperglycaemia, lipolysis and hyperlipidaemia which are characteristics of uncontrolled diabetes mellitus [23].

The observed blood sugar levels of both treated and untreated groups as well as the control group showed a highly significant difference ($p<0.01$) as shown in Table 2. It revealed that the blood sugar levels of the diabetic groups of experimental Wistar rats treated with insulin were significantly lower ($p>0.05$) than those treated with aqueous extract of cinnamon. This supports the earlier claim by Saima and Aishat [17] that aqueous extract of Cinnamon had been shown to potentiate the hypoglycaemic effect of insulin through up-regulation of glucose uptake in cultured adipocytes of rats.

Most prominent feature in the untreated STZ-induced diabetic group of Wistar rats (Fig. 2) was the distortion of hepatic architecture which radiates from the central veins with the presence of vacuoles which may have resulted from fat infiltration. There is halo and distortion of cell nuclei in this group. However, it was observed that the cyto-architecture was normal in the control group as well as groups that were made diabetic with STZ and subsequently treated with insulin and aqueous extract of Cinnamon respectively. The normoglycaemic state that was restored following treatment of diabetes with these agents could be responsible for this normal histologic picture.

CONCLUSION

Diabetes Mellitus is one of the major chronic diseases in all populations[24]. The currently available agents for treatment of diabetes are expensive, not easily accessible and have several side effects[25]. Thus a large number of studies are involved to find natural hypoglycaemic and anti-lipedemic products as alternatives to the synthetic ones.

REFERENCES

1. Kim SH, Hyun Sh, Choung SY. Antidiabetic effects of Cinnamon Extract on blood glucose in db/db mice. *J. Ethnopharmacol.* 2006; **104**:119-123.
2. Chait A and Brunzell JD. Diabetes, lipid and atherosclerosis.' *in D.Le Roith, S. I Taylor and J.M Olofsky (Edu). Diabetes Mellitus.*1996; 467 – 469.
3. Petal M and Rybezynski P. Treatment of non-insulin dependent diabetes mellitus.' *Expert opin. Investing. Drugs.* 2003; **12(4)** : 623-633.
4. Fuller JH, Shipley MJ, Rose GH, Jarrett RJ and Keen H. Coronary – heart disease risk and impaired glucose tolerance : The white tall study. *Lancet.* 1980; **1**: 373 – 1376.
5. Muller NA, Faloona GR and Unger RH. Hyperglucagonemia in diabetic Ketoacidosis. Its prevalence and significance. *American Journal of clinical medicine.* 1973; **54**: 52 – 57.
6. Bergman, RN. Non-esterified fatty acids and the liver: why is insulin secreted into the portal vein. *Diabetologia.* 2002; **43:**. 946-952.
7. Wolfe, R.R., and Volpi, E. Insulin and protein metabolism. In Handbook of physiology: the endocrine pancreas and regulation of metabolism. L.S. Jefferson and A. Cherrington. editors. *Oxford University Press.* New York. 2001; 735–757.
8. Gray AM and Flat PR. Pancreatic and extra-pancreatic effect of the traditional anti-diabetic plant medicago sativa (Lucerne). *British Journal of Nutrition.* 1997; **78**: 325 -334.
9. Anderson RA and Broadhurst CL. Isolation and characterization of Polyphenol type-A polymers from cinnamon with insulin – like biological activity. *Journal of Agricultural and food Chemistry.* 2004; **52**: 65 – 70.
10. Bailey CY and Day C. Traditional plant medicines as treatment for diabetes. *Diabetes care. 1989;* **12**: 553 – 564.
11. Chung TH. Kerean flora (Herb part), In: Anti-diabetic effect of Cinnamon extract on Blood glucose in db/db mice. *Journal of Ethnopharmacology. 1994;* **104**: 119 -123.
12. Abdul Rahim Al Jamal. Effects of cinnamon on Blood glucose and lipids level in diabetic patients (type1). *African Journal of Biochemistry Research. 2009;* **3(5)**: 181-184.
13. Soheir NR, Amal MH and Hess A. Antidiabetic effects of Cinnamon powder and Cinnamon aqueous extract on serum glucose of rats. *International journal of food Nutrition and public Health.* 2010; **3(2)** 183-197.
14. Wijesekera RO. Historical Overviews of the Cinnamon industry. *CRC Critical Reviews in food science and Nutrition. 1978;* **10**: 1 – 30.
15. Hiromu K, Icatudi O and Nenokidu H. Constituent of the essential oil from cinnamomum loureirri Nees. *Reports of the*

scientific Research Institute. 1974; **10**: 47 –50.
16. Jarvull - Taylor KJ, Anderson RA and Graves DJ. Functions as a mimetic for insulin in 3T3 –LI adipocytes'. *Journal of American College of Nutrition. 2001;* **20** : 327 -336.
17. Saima Mahmonnd, Aisha Talat, Sabiha Karim, Rukhshan Khurshid and Azam Zia. The effect of Cinnamon extract on Blood glucose level and lipid profile in Alloxan - induced diabetic Rats. *Pakistan Journal of physiology. 2011;* **7(1)**: 13 – 16.
18. Ranjbar A, Gharmeinezhad S, Zamani H, Malekirad AA, Baiaty A, Mohammadirad A and Abdullahi M. Antioxidative stress potential cinnamonum zeylanicum in humans: a comparative cross-sectional clinical study. *Therapy. 2006;* **3**: 113 – 117.
19. Onderoglu S, Sozer S, Erbil KM, Ortac R and Lermioglu F. The Evaluation of long term Effects of Cinnamon bark and Olive leaf on toxicity induced by streptozotocin admin to rats. *Journal Pharm. Pharmacol. 1999;* **51(11)**: 1305 – 1312.
20. Broadhurst Cl, Polansky MM and Anderson RA. Insulin – like biological activities of culinary and medicinal plant aqueous extracts in vitro. *J. Agric Food Chem. 2000;* **48(3)**: 849 -852.
21. Khan A, safdar M, khan mma, khahak kN and Anderson RA. Cinnamon improves glucose and lipids of people with type 11 diabetics. *Diabetics case. 2003;* **26**: 215- 3218.
22. Guide for the care and use of laboratory animals. Published by the US National Institute of Health (NIH publication No 85-23). 1996.
23. Chait A and Brunzell JD. Diabetes, lipid and atherosclerosis. *in D.Le Roith, S. I Taylor and J.M Olofsky (Edu). Diabetes Mellitus*. 1996; 467 – 469.
24. Wild S, Roglic G, Green A, Sicree R and King H. Global prevalence of diabetes : Estimates for the year 2000 and projections for 2030. *Diabetic care. 2004;* **27**: 1047- 1053.
25. Ramesh, B and K. V. Pugalendi. Antihyperlipidaemic and antidiabetic effects of receptors causes hyperphagia and insulin resistance in rats. *Nat. Neurosci.* 2005; **5:** 566-572.