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Antidiabetic effect of Standardized Extract of *Avena sativa* seeds against streptozotocin affected diabetes in rats

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ABSTRACT

Oats also known as Avena sativa prone to best developments in temperate regions and found in North West Europe, Central America and even Iceland also. Oats are annual plant, and might be set either in fall (for late summer harvest) or within the spring (for early fall harvest) and is employed as Neutraceuticals and historically for a variety of health disorders together with cardiovascular disease, upset, nervous prostration, insomnia, and helplessness of the nervousness. They are thought-about as medicinal drug, antitumor, cyanogenic, demulcent, diuretic, neurotonic, stimulant and inhibitor. During this work, the results of seeds of cereal oats. On fast glucose levels in streptozotocin evoked diabetic rats were examined reciprocally with its effects on the supermolecule profile in-vivo. The oral aldohexose tolerance check disclosed that animals treated with seeds of ethanolic extract of Avena sativa showed vital decreases in their plasma glucose level compared with management cluster treated with gum acacea.. Avena sativa seeds induced noteworthy reduction in liquid body substance aldohexose levels in streptozotocin diabetic rats once fourteen and twenty one days, reducing the aldohexose concentration by 38.6% and 47.0%, severally, once administered at three hundred mgkg-1. When administered to streptozotocin induced diabetic rats at three hundred mg kg-1 Avena sativa Seeds had strong effects on their lipid profile by significantly decreasing total lipid, triglyceride and cholesterol. This pharmacological activity investigation has confirmed that Avena sativa seeds confers moderate defense against diabetes in-vivo.

In summation, the potential of Avena sativa seeds to reduce triglyceride and total cholesterol levels while increasing high density lipoprotein may contribute to its beneficial effects in diabetic rats.

1. Introduction

The illness hassle associated to polygenic disorder is high and growing in each country, fuelled by the worldwide rise within the incidence of fatness and unhealthful lifestyles. The illness burden associated with polygenic disorder is high and growing in each state. The most recent estimates prove a worldwide prevalence of 382 million folks with polygenic disorder in 2013, expected to rise to 592 million by 2035 (Expert Committee report, 2003) diabetes (DM), a metabolic syndrome and characterized by symptom and inadequate secretion or action of hypoglycaemic agent.

As per International polygenic disorder Federation's (IDF) estimates, eightieth of the diabetic population across the world are from low and middle financial gain countries by 2030 **(Baynes H W, 2015)** diabetes is characterized by symptom, glucosuria, negative balance and typically ketosis **(Tripathi KD ,2003)** principally folks plagued by diabetes either type one pol ygenic disorder (which is immune-mediated) or type 2 DM (formerly called non insulin dependent polygenic disorder mellit us) **(S. Asgary et.al., 2012)** DM could be a chronic metabolic illness which might be clinically suspected by the onset of characteristic symptoms like nephropathy, polydipsia, polyphagia and unresolved weight loss **(Gandhi GR and P. Sasikumar, 2012)** cereal oat normally called Oats Groats, Haber, Hafer, Avena, Straw, Oatmeal, could be a species of cereal grain adult for its seed. It is one with all the necessary healthful herbs of the Gramineae From ancient time plant species are getting used within the treatment of varied diseases. ***A. sativa L.*** is yearly grass concerning 1.5 meters tall; calms tufted or solitary, raise or twisted at the underside, smooth. The leaves are non-articulate, green, and also the sheaths rounded on the back; the leagues are blunt and membranous. The inflorescence coul d be a diffuse racemewith 2–3 florets, all bisexual or the distal one or 2 is also reduced and male or sterile; glumes sub-equal 7–11 patterned; longer glumes 17–30 mm; lemmas 7–9 veined, either divided or with a bridle at their apex; lowest lemma is 12 25 millimetre. The rachillas of the cultivated oat do not part at maturity (that of many weed species does). Its lemmas are seldom awed. The grain is sort of surround by the exhausting lemma and palea. Seed size varies with the cultivar; it's normally concerning thirty,000 seeds per metric weight unit crop **(Gibbs Russell et.al., 1990)** All the medicine (I. e. insulin, sulphonylureas and biguanides) are related to adverse impact and powerless to manage metabolism effectively. Supervision of diabetes with agents devoid of any side effects is still a challenge to the medical system. At that place is increasing curiosity in herbal remedies due to these causes. But still only approximately 5 % of the total plant species has been thoroughly examined for its safety and efficacy.

Current study reflects the application of medicinally active plant Avena sativa as antidiabetic, antioxidant and neutraceutical **(Pradeepa R, Mohan V, 2002; Coffman, F. A.,1977).**

Plants play main role in the innovation of new therapeutic agents and much awareness has been acknowledged in this regard as sources of biologically active substances **(Chikhi I et.al., 2014)** Avena sativa is one such plant selected for the study. The different sections of the plant are used traditionally as medicine due to good therapeutic values in the present study anti diabetic activity of the ethanolic seed extract (ASE) of Avena sativa has been ascertained in streptozotocin induced diabetic rates **(Chopin, J. J. et.al., 1977).**

2. Materials and Methods

2.1 Chemicals required:

All chemicals and reagents used were of analytical grade and were obtained from following indicated commercial sources. Thiobarbituric acid, nitroblue tetrazolium (NBT, Loba chemie, Mumbai), 5,5-dithio bis-2- nitrobenzoic (DTNB), reduced glutathione (GSH), streptozotocin from (SISCO Research Lab Mumbai). Glibenclamide (Daonil ^{TM,} Sanofi India Ltd Pune, India), were purchased from local medical store , Amethi, India.

2.2 Plant Materials:

The seeds of *Avena sativa* L. (Poaceae) were procured commercially from Amethi local market. The seeds were authenticated by Dr. Sayyada Khatoon, Taxonomist, National Botanical Research Institute (NBRI), Lucknow, India vide specification no. NBRI-SOP-202 dated 21.01.2013.

2.3 Experimental Animals:

Sprague-Dawley rats (100-150g) and mice (25-30 g) of either sex were used for the study. They were kept under controlled conditions of temperature 27± 2ºC and relative humidity 44-56%, light/dark cycles of 12 hours respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Hindustan Liver Ltd. Mumbai, India) and the food was withdrawn 18-24 h before the experiment though water was permitted *ad libitum*. All experiments were performed in the morning accordance with the recent guidelines for the care of laboratory animals and the ethical procedure for investigations of experimental pain in conscious animals **(Zimmerman M, 1983)**. The protocols were approved by Institutional Committee for Ethical use of Animals and Review Board (Reg. No.1045/Ere/07/CPCSEA). All the experiments were carried out in accordance with the institutional committee guidelines RRSCOP, Amethi, India.

2.4 Preparation of Extract:

The *Avena sativa* seeds (2 kg) coarse powder dried under the control conditions and powdered materials was extracted with petroleum ether in soxhlet apparatus to remove fatty substances, the marc was further exhaustively extracted with chloroform and finally with 60 % ethanol. The extract was separated by filtration and concentrated and dried in hot air oven to obtained light brownish solid residue.

2.5 Phytochemical Screening TLC and HPTLC Analysis

The ethanolic extract of *Avena sativa* (ASE) were analyzed for occurrence of Lipids, alkaloids, steroids and saponins, flavonoids, and triterpenoid saponins, as described by Trease and Evans,1989 **(Trease GE *et.al.,* 1989; Harborne JB, 1993)** TLC analysis was processed on activated silica gel plates in by using mobile phase chloroform: methanol (96:4) and detection was done by Iodine vapour which showed yellow- brown spot with white background. HPTLC analysis was processed on preactivated (100ºC) Aluchrosep silica gel 60F254 HPTLC plates (S.D.fine-chem Ltd, Mumbai, India) together with quercetin and HPTLC plates were eluted in solvent system toluene : ethyl acetate : formic acid (5:4:1) for phenols. Then After development, the plates were dried and densitometrically scanned at wavelength 366 nm (Win Cats software, CAMAG, Switzerland).

2.6 Physicochemical parameters

All parameters were applied on seeds physicochemical analysis i.e., percentage of ash values, moisture content and extractive values, were performed according to the official methods given in Indian Pharmacopoeia, 1996 and the WHO guidelines on quality control methods for medicinal plant materials **(WHO/QCMMPM guidelines, 1992)**

2.7 Acute Toxicity Studies:

The adult male albino mice selected for acute toxicity study. The 60% ethanolic extract of *Avena sativa* were taken at various doses levels (100, 200, 400, 800, 1000, 1500, 2000 mg/kg body wt.) dissolved in 1 % carboxymethyl cellulose orally to five mice for every dose level. The control animals received 1 % carboxymethyl cellulose in distilled water (10 ml/kg) orally. The animals were observed continuously for two hour and then rarely for further four hours and finally any mortality, behaviour (gross behaviour, general motor activity, writhing, seizure, response to tail pinching, pupil size, fecal output, water intake, feeding behavior, sedation *etc*.) of the animals and any other toxic symptoms also observed for 72 hours and the animals were kept under observation up to 14 days (OECD 423) **(Zhang CF *et.al.,* 2001)**

2.8 Experimental Induction of Diabetes

Overnight fasted Rats (given water *ad libitum*), were administered a single high dose of streptozotocin (50 mg/kg b.w.) interperitonially (i.p.) prepared in citrate buffer (0.1 M, pH 4.5). After 96 hour (h) fasting blood glucose level were checked with glucometer (Dr. Morepen, Morepen Lab. Ltd.,New Delhi). Rat with fasting blood glucose level 200mg/dl and above were selected for further experiments.

2.9 Oral glucose tolerance test

Oral glucose tolerance tests **(Turner RA, 1965)** were performed in overnight-fasted (18 h) normal rats. Rats were divided into two groups of six rats each (n=6) and these were administered drinking water or *Avena sativa* extract (ASE) (300 $mgkg^{-1}$) in water orally, respectively. Rats were loaded with glucose ($2gkg^{-1}$) 30 min after administration of ASE. Blood was withdrawn from the retro orbital sinus under ether inhalation at 0, 30, 60 and 120 min after glucose administration and serum glucose level was estimated by enzymatic glucose oxidase-peroxidase method using a glucose diagnostic kit (Sigma-

Aldrich, Mumbai, India), in which glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of enzyme peroxidase oxidizes phenol which combines with 4- aminoantipyrine to produce a red-coloured quinoneimine dye. The intensity of the red colour so developed was measured at 505 nm and was directly proportional to the glucose concentration **(Bonner-Weir S ,1988)**

2.10 Experimental design

After induction of diabetes, the rats were divided into five groups.

Group 1 control rats, received vehicle solution (2% gum acacia).

Group 2. diabetic control, received streptozotocin (50 $mgkg^{-1}$, i.p.).

Group 3 diabetic rats treated with ASE 100 $mgkg^{-1}$ in 2% gum acacia.

Group 4 diabetic rats treated with ASE 300 $mgkg^{-1}$ in 2% gum acacia.

Group 5 diabetic rats treated with Glibenclamide 1 $mgkg^{-1}$ in aqueous solution.

The vehicle and drugs were administered orally using an intragastric tube every day for three weeks. After three weeks of treatment, the rats were fasted whole night and blood samples were analyzed for serum glucose concentration. **(Trinder P,1969)**

2.11 Plasma lipid profile

The serum cholesterol level was estimated by the (Wybenga DR *et.al*.) method using a cholesterol diagnostic reagent kit (Oscar Medicare Pvt. Ltd., New Delhi, India). Cholesterol reacts with a hot solution of ferric perchlorate, ethyl acetate and sulphuric acid (cholesterol reagent) and gives a lavender-coloured complex which is measured at 560 nm in visible spectrometer. The total lipid was estimated by the Phosphovanillin method using a Total Lipids diagnostic reagent kit (DiaSys Diagnostics India Pvt. Ltd., Mumbai) **(Bonner-Weir S ,1988)** Lipids formed a coloured complex when treated with Phosphovanillin in sulphuric acid solution, and the absorbance at 520 nm was proportional to the amounts of total lipids present. Triglyceride was analysed by the glycerol phosphate oxidase method using Triglyceride diagnostic kit (Angstrom Biotech Pvt. Ltd., Gujrat) **(Trinder P,1969)** Triglycerides in the sample were hydrolysed by microbial lipases to glycerol and free fatty acids .Glycerol was phosphorylated by adenosine 5 triphosphate (ATP) to glycerol-3-phosphate (G-3-P) in a reaction catalysed by the enzyme glycerol-kinase (GK). G-3-P was oxidized to dihydroxyacetone phosphate (DAP) in a reaction catalysed by the enzyme glycerol phosphate oxidase (GPO). In this reaction H_2O_2 was produced in equimolar concentration to the point of triglycerides present in the sample. H_2O_2 reacted with 4- aminoantipyrine (4-AAP) and 4-chlorphenol in a reaction catalysed by peroxidase (POD). The result of this oxidative coupling was a chinonimine, a red coloured dye. The absorbance of this dye in solution was proportional to the concentration of triglycerides in the sample.

2.12 Statistical analysis

The statistical analysis of all the pharmacological studies was carried out using Graph pad prism. The *in-vivo* data were presented as mean ± s.e.m. for six rats and as described in the table 2 & 3 legends for *in vitro* experiments. Differences between treatments were assessed using analysis of variance, followed by paired-*t* test for multiple comparisons. Differences were considered significant when $P<0.05$.

3. Results and Discussion

3.1 Physicochemical Studies

Ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The alcohol soluble extractive was high in seeds of *Avena sativa*. The results of physicochemical constants of the drug powder are presented in (Table 1)

Table 1
Physicochemical studies of *Avena sativa*

Parameter	Result
Ash value	8.7 %
Acid insoluble Ash	2.65% w/v
Water soluble Ash	3.47% w/v
Water insoluble Ash	3.36% w/v
Moisture content	3.6% w/w
Extractive value(water soluble)	14.89 w/v
Extractive Value (alcohol soluble)	6.89 w/v
pH of 1% suspension	6.23 w/v

3.2 Phytochemical Analysis

Phytochemical results showed the presence of alkaloids, carbohydrates, flavonoids, tannins and phenolic compounds. Quantitative HPTLC determination showed the presence of 0.18112% w/w of quercitrin (a flavonoid) in ethanolic extract of *Avena sativa* seeds (ASE) (**Figure 1& 2**).

3.3 Acute toxicity studies

Acute *in-vivo* toxicity studies revealed the non-toxic nature of *Avena sativa* seeds (ASE). There were no mortality or any toxic observations found at the doses selected up to the end of the study period.

3.4 Antidiabetic activity

The oral glucose tolerance test revealed that animals treated with seeds of ethanolic extract of *Avena sativa* seeds (ASE) showed significant reductions in plasma glucose level compared with control group treated with gum acacia. Streptozotocin has been commonly used for inducing type I diabetes in various animals by promoting degeneration and necrosis of pancreatic *β*-cells,(Merzouk H *et.al.*,2000) Diabetes induced by streptozotocin in rats was established by the existence of high fasting plasma glucose levels (**Table 2**, Diabetic Control) **(Figure 3)**. *Avena sativa* seeds (ASE) induced noteworthy reduction in serum glucose level in streptozotocin diabetic rats ($P< 0.001$) after 14 and 21 days, reducing the glucose concentration by 38.6 and 47.0%, respectively, when administered at 300 mgkg-1 (**Table 2**). The speedy onset of the glucose-lowering effect of *Avena sativa* seeds (ASE) in diabetic rats was unlikely to be related to *β*-cell neogenesis. However, it was expected that not all *β*-cells were damaged by the single streptozotocin dose of 50 mgkg−1 used in these experiments, since glibenclamide, a sulphonylurea

that stimulates insulin secretion by acting at *β*-cell ATP-sensitive K^+ channels, restored blood glucose levels to the normal range in streptozotocin diabetic rats (**Table 2**). Thus, *Avena sativa* seeds (ASE) may also have exerted its glucose lowering effects by directly stimulating insulin secretion from *β*-cells that had not been destroyed by streptozotocin treatment. It is possible that, in the *in-vivo* experiments, residual *β*-cells following streptozotocin-induced diabetes might be stimulated to secrete insulin, and so lower the level of fasting blood glucose. In patients with severe hypertriglyceridaemia, especially where diabetes is accompanied by genetic hyperlipidaemia, therapy with lipid lowering drug is required. When administered to streptozotocin induced diabetic rats at 300 mgkg−1 *Avena sativa* seeds (ASE) had strong effects on their lipid profile by significantly (*P*< 0.001) decreasing total lipid, triglyceride and cholesterol (**Table 3**) **(Figure 4)**.

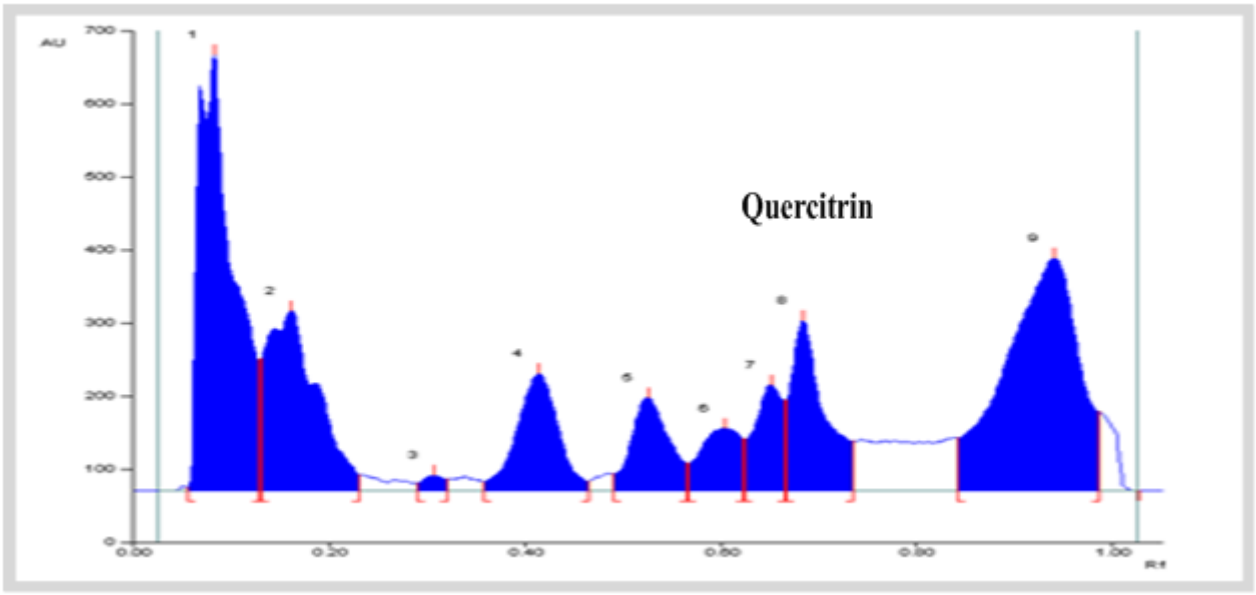

Figure 1: HPTLC finger print profile of ethanolic extract of *A.sativa (ASE).*

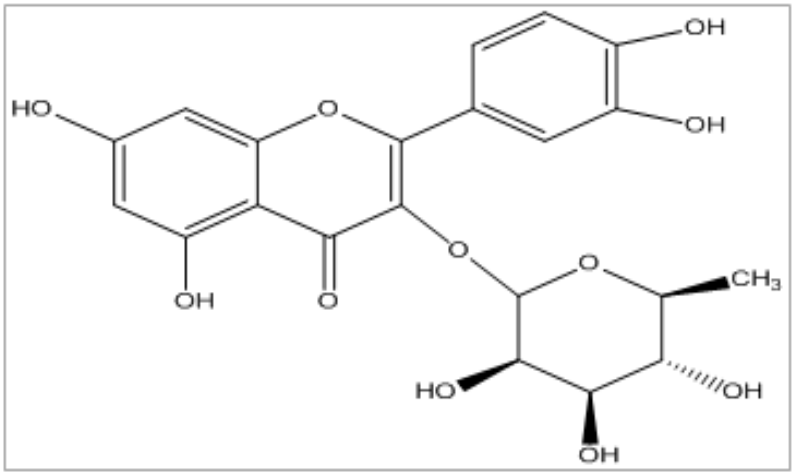

Figure 2: Quercitrin

Table 2
Effects of the ethanolic extract of *Avena sativa* (ASE) on serum glucose levels in streptozotocin induced diabetic rats

Groups	Blood glucose (mmol/l)			
	Day 0	Day 7	Day 14	Day 21
Normal Control	04.59±0.57	04.39±0.69	04.47±0.35	04.26±0.52
Diabetic Control	08.88±1.24	09.13±1.56	08.76±0.51	09.08±0.23
ASE 100	07.18±0.12	06.88±0.16[a]	06.57±0.19[b]	06.38±0.18[b]
ASE300	07.02±0.08	06.72±0.14[b]	06.36±0.18[c]	06.18±0.13[c]
Glibenclamide	07.1±0.13	05.92±0.14[b]	05.63±0.06[b]	05.54±0.09[c]

The values of blood glucose in the table represent the means ± s.e.m. for six rats per group upon treatment with normal saline, ASE and glibenclamide. *P* values were calculated based on the paired-*t* test. [a]*P*<0.05, [b]*P*<0.01 and [c]*P*<0.001 compared with diabetic control group.

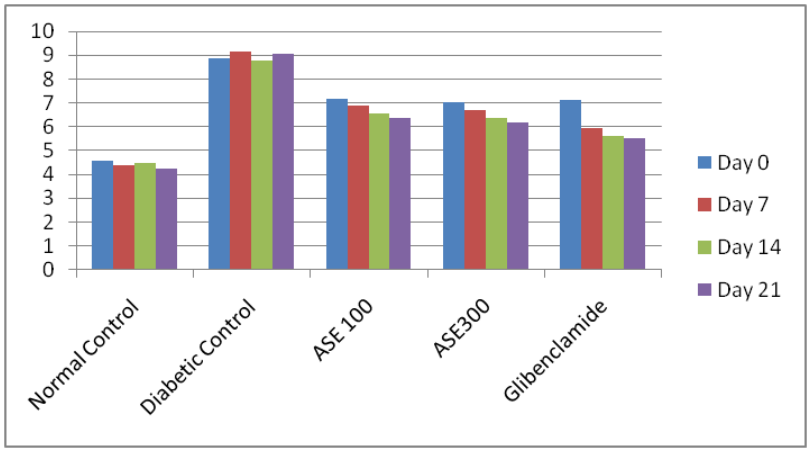

Figure 3: Effects of the ethanolic extract of *Avena sativa* (ASE) on serum glucose levels in streptozotocin induced diabetic rats

Table 3
Effect of the ethanolic extract of *Avena sativa* (ASE) on the level of serum total lipids, triglycerides and cholesterol in streptozotocin-induced diabetic rats

Group	Total lipids ($mgdL^{-1}$)	Triglycerides ($mgdL^{-1}$)	Cholesterol ($mgdL^{-1}$)
Normal Control	081.23±1.74	71.56±1.46	73.95±2.38
Diabetic Control	148.37±0.40	116.89±1.23	161.23±2.75
ASE100	111.16±2.47^{c}	92.14±1.81^{c}	99.75±2.75^{c}
ASE300	089.74±2.51^{c}	67.48±1.46^{c}	72.16±2.21^{c}
Glibenclamide	089.87±1.39 ^{c}	73.96±1.86^{c}	82.11±0.58^{c}

The values of lipid profile in the table represent the means ± s. e. m. for six rats per group upon treatment with normal saline, ASE and Glibenclamide. *P* values were calculated based on the paired-t-test. $^{c}P<0.001$ compared with diabetic control group.

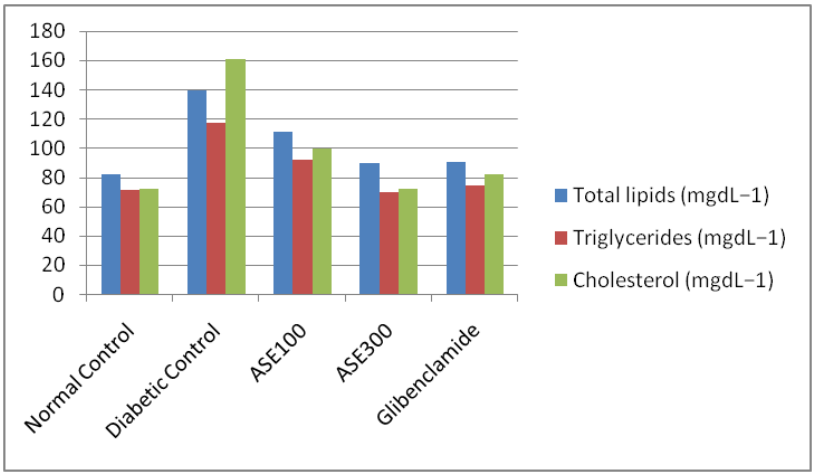

Figure 4: Effect of the ethanolic extract of *Avena sativa* (ASE) on the level of serum total lipids, triglycerides and cholesterol in streptozotocin-induced diabetic rats

4. Conclusion

This pharmacological activity of evaluation study of Oats has confirmed that Avena sativa (ASE) confers moderate defense against the diabetes in-vivo.

In addition, the possibility of Avena sativa to reduce triglyceride and total cholesterol levels while increasing high density lipoprotein may contribute to its beneficial effects in diabetic rats.

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