Research Article

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Journal of Clinical and Molecular Endocrinology
ISSN 2572-5432

2021
Vol. 6 No. 3 :42

DOI: 10.36648/2572-5432.6.3.42

An Assessment of Source and dose-dependent Diabetes Ameliorating activity of Ethanolic Extract of Nigella sativa on Alloxan-Induced Diabetic Rat Model

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Abstract

Metabolic disorder diabetes results from an alteration of the secretion or action of insulin. *Nigella sativa* is a traditionally used specimen since ancient times. We aimed to investigate the hypoglycemic potential of ethanolic extract of *Nigella sativa* seed powder solution both in a dose and source-dependent manner as well as to fathom out its safety profile so that this plant can be used to ameliorate diabetes. Diabetes was induced in the rat model via intraperitoneal injection of alloxan (150 mg/kg). Ethanolic extract of **T.** *Nigella sativa* was administered to rats' belonged to different groups. Blood glucose levels were assessed periodically and the safety profiles were evaluated through assessment of SGOT, SGPT, creatinine, and lipid profiles after sacrificing the animals. It has been evidenced that *Nigella sativa* possesses anti-diabetic activity. Furthermore, the extract is capable of reversing the disturbed pathological state towards a healthy status. Besides, these therapeutic consequences possess dose-dependent potentiality ($p>0.05$), further a noteworthy source dependent ($p> 0.01$) response were experienced. It may confer that the inconsistency associated with the remedial impacts between 2 same doses belonged to two distinct sources are due to accuracy of lab-based preparation, geographic area of cultivation, and also the season of collection. Apart from that, the visual and statistical inspections have evidence that the medium and the high dose are imparting almost indistinguishable therapeutic effects. Presumably, the reason lies beneath the receptor saturation issue.

Keywords: Nigella sativa, alloxan, hypoglycemic effect, dose dependency, source dependency, safety profile

Received: April 16, 2021; **Accepted:** April 30, 2021; **Published:** May 07, 2021

Citation: Akter D, Rokeya CA, Tahsin Md.R, Haque E, Sultana A, et al (2021). An Assessment of Source and dose-dependent Diabetes Ameliorating activity of Ethanolic Extract of Nigella sativa on Alloxan-Induced Diabetic Rat Model. J Clin Mol Endocrinol. 2021, 6:3.42

Introduction

Type 1 Diabetes mellitus (T1DM), is a chronic autoimmune disease which have stemmed as a leading global health problem and affects people of all ages, genders and races [1]. According to an estimation, around 5%–10% of adults worldwide are afflicted by this disorder and this will increase 20%–69% in the next 20 years [2,3]. Respectively, the outbreak of diabetes among those aged between 20 to 79 years may be expected to increase 7.7%, constituting 439 million by the year 2030 [4,5]. It is caused by complete or partial insulin deficiency which results in hyperglycaemia [6]. This elevation in blood glucose levels has stimulated the production of reactive oxygen species (ROS), which cause cellular damage that promote the progression of acute and chronic complications including hypoglycemia, ketoacidosis, nonketotic hyperosmolar coma, diabetic retinopathy, nephropathy, autonomic neuropathy, microangiopathy, various organ failure and infections [7-10]. Moreover, Diabetes mellitus can also be conjoined with cardiovascular risk factors such as hypertension, dyslipidemia and obesity [11].

Diabetes cannot be mitigated completely rather it must be kept under tight management [12]. This intricated condition might be controlled by changing diet, sedentary lifestyles and medications [13]. The most commonly used medicine to control diabetes include insulin and its derivatives, glucagon-like peptide-1

receptor agonists, thiazolidinediones (TZDs), sulfonylureas, amylin analogues, biguanides, and glucosidase inhibitor [14-17]. Current medicaments for the treatment of type 2 diabetes mellitus have detrimental effects, and sometimes, they are reported for being ineffective in patient with chronic diabetes [18]. Furthermore, no medication can intensify both insulin sensitivity and secretion simultaneously.

Medicinal plants have attained wide attention from scientists and have been deemed to be a beneficial adjuvant agent as an oral antidiabetic and hypolipidemic drug, mostly in developing countries, due to their integrated effects, rare or no side effects and lower cost. [19,20]. Besides that, Nigella sativa has also been thought to be safer among 1000 different antidiabetic medicinal plants compared to oral antidiabetic drugs [21]. Nigella sativa is an annual herbaceous plant spices, belonging to the family Ranunculacea which can be found mostly in Middle Eastern countries including Pakistan, India, Italy, Indonesia and Afghanistan [22]. It is most commonly known as "black seed", "black cumin" or "kalajeera". Different forms of Nigella sativa like extract, oil, and powder have been employed in traditional medicine to treat several illnesses such as fever, diarrhea, bronchitis, cough, hemorrhoids, gastrointestinal, hepatitis and tapeworm disease [23-25]. It is also known as an immunity enhancer. Nigella sativa extract has been demonstrated to possess immunopotentiating [26], antioxidant [27], antitumoral [28], antidiabetic [32], anti-proliferative [30], antimicrobial [30], antiasthmatic [33], antihypertensive [32], antiparasitic [31], anti-fertility [33], hypolipidemic [29], anti-inflammatory [29], and anti-pyretic [29] activities. Screening for unique phytochemical constituents from Nigella Sativa has earned researcher's attention because of its ameliorative effects. The ameliorative effects of Nigella Sativa are mainly conferred to thymoquinone, which is one of the major bioactive compounds that was unveiled to have a defensive effect against diabetes [34]. Previous studies stated that thymoquinone introduced a marked decrease in Fasting Blood Glucose level and a noticeable increase in insulin levels in rats [35]. Besides thymoquinine, the other compounds, namely thymol, thymohydroquinone, dithymoquinone, nigellone, alpha-hederin, flavonoids, alkaloids, volatile (0.40%–0.45%) and non-volatile (32%–40%) oils, carbohydrates (31.0%–33.9%), protein (16.00%– 20.85%), fibre (5.50–7.94%), tannins, saponins, minerals such as iron, potassium, magnesium, calcium, zinc and copper (1.79%–3.44%), vitamin A and C, niacin, pyridoxine, thiamine, folate and fatty acids were also found to have therapeutic properties [36,37]. Additionally, kalonji was presented to have no caustic side effects or toxicological effects in both human and animal models [38].

The modern drugs used in the management of diabetes is heavily overpriced, and also burden and unreachable for mass population. The aim of our current study is to investigate the antidiabetic and hypolipidemic effect of Nigella sativa in a dose and source dependent manner as well as relative adverse effects and safety profile study on liver and kidney in alloxan-induced diabetic rat model.

Method Materials

We used highest analytical grade chemicals in our current study. Dried *Nigella sativa* seeds were bought from Allahrdan Shop, Banasree, and Dhaka. Humalyzer 3000 (Semi-Automated Clinical Chemistry Analyzer of Medigroup Asia limited, Combodia) was employed to measure the blood parameters of rodents. Glucometer of Alere GI of Alere Inc, USA was instituted from Shahbag, Dhaka, Bangladesh. All blood parameter analyzing kits were received from Plasmatic Laboratory Product Limited. A chemical agent (alloxan) was purchased from Sigma Aldrich, Germany.

Extraction Procedure

Firstly, Nigella sativa seeds were thoroughly washed and dried in sunlight for few days. Afterwards, the dried seeds were crushed into powder. Then, the dry powdered materials were soaked in methanol and kept for 14 days with occasional vigorous stirring and shaking. Subsequently, the extract was filtered by using Whatman No.1 Filter paper. To reduce the volume from a rotary evaporator at low temperature and pressure the filtrate liquid was taken for the next step.

Experimental Design and Animal Handling

Wister albino thirty adult male rats were obtained from the animal unit, Jahangirnagar University, Department of Pharmacy, Dhaka, Bangladesh. They were incarcerated individually in stainless steel cages at 12±1 h light/dark cycle under the controlled temperature (25◦C) in the Institute of Nutrition & Food Science, University of Dhaka. The rats were given with a standard pellet diet and water ad libitum. Before initiating the analysis, the rats were in housed there for acclimatization. The bodyweight of each rat has been weighed afterward. The animals were divided into 6 groups where an even division of rodents as per their body weight has been taken place, and each group included 5 rats.

Group 1: Normal Control

Group 2: Diabetic Control

Group 3: Low Dose (100 mg/kg body weight)

Group 4: Medium Dose (400 mg/kg body weight)

Group 5: High Dose (750 mg/kg body weight)

Group 6: Commercial Preparation (400 mg/kg body weight)

The rats were fed normal food and water twice daily in the first two weeks without initiating diabetes. A chemical agent, alloxan (150 mg/kg body weight), was injected into all groups via intraperitoneal route for diabetic induction except normal control group on the 14th day. After 72 hours, the blood sugar level was scrutinized. It has been observed that diabetes was incited in all rats associated to groups 2-5 and treatment was commenced on the 18th day, which was continued for twenty-eight days. The blood glucose level was examined once in every week. The doses were given by oral route of administration.

Statistical Analysis

The discoveries of all study parameters associate to several groups were delineated as mean±SD. "One Way Anova Test" of SPSS 16" software was used to explore the inter-group discrepancies in results to trace the statistical significance. Here, the statistical significance level was set at a 'p' value of

p<0.05. On the other hand, high statistical significance was set at 'p' value of p<0.01. In terms of results, the inter-group differentiability was thought statistically significant and highly significant when the p-value was seen less than 0.05 and 0.01 respectively.

Results

Change in body weights

The pre-treatment & post treatment body weight (gram) of rats belonged to different groups are shown in Figure 1

Change in blood glucose level

The blood glucose level (mmol/dl) of all test group from day 1 to day 42 are expressing below in mentioned graph in Figure 2.

Safety Profile Study (Liver function test)

The SGOT level of all rats belonged to 6 groups are denoting the condition of liver is shown in below graph Figure 3.

The level of SGPT of all rats that belonged to 6 groups is expressing the condition of liver are expressing via the blow shown graph Figure 4.

Safety Profile Study (Kidney functioning Test)

The below mentioned values concerning the level of Creatinine (md/dl) of rats belonged to 6 group as a requirement of measuring the kidney functioning test are presenting in Figure 5

Safety Profile Study (Lipid Profile)

The level of Total Cholesterol Level of all rats belonged to 6 groups are expressing in below Figure 6.

Safety Profile Study (Lipid Profile)

The level of HDL level (mg/dl) of all rats belonged to 6 groups are presenting in below drawn graph, Figure 7.

Safety Profile Study (Lipid Profile)

The below mention values regarding the level of LDL level (mg/dl) of rats belonged to 6 groups are presenting in below, Figure 8.

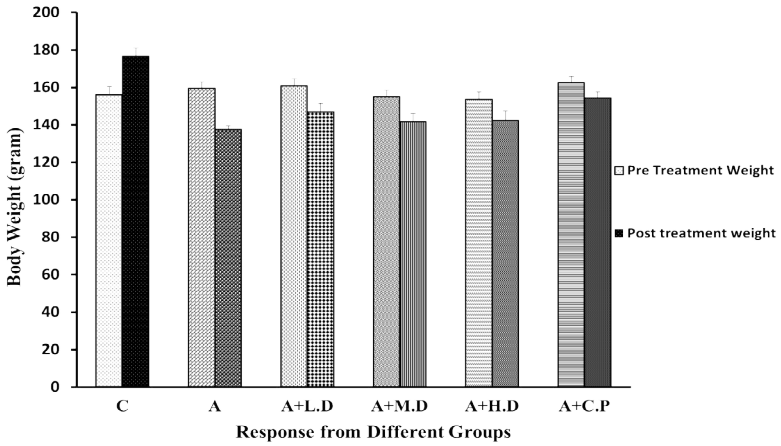

Figure 1 Comparision between the average body weight (mean±standard deviation) of rats belong to 6 groups before starting the experiment and just before sacrifice.

C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation

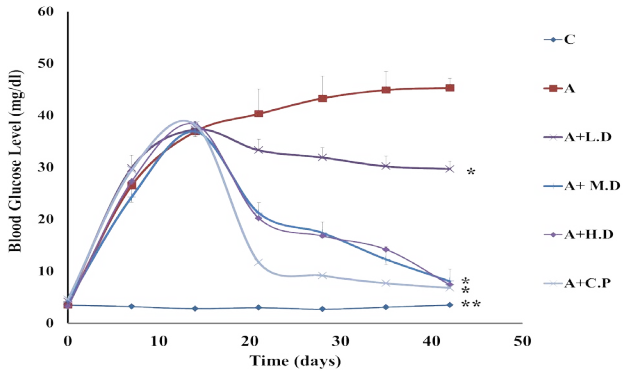

Figure 2 Blood glucose level of six groups from day zero to day forty-two. The data were expressed as mean± standard deviation.

* Expresses the significant change,

** Expressing the high significant change. C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation

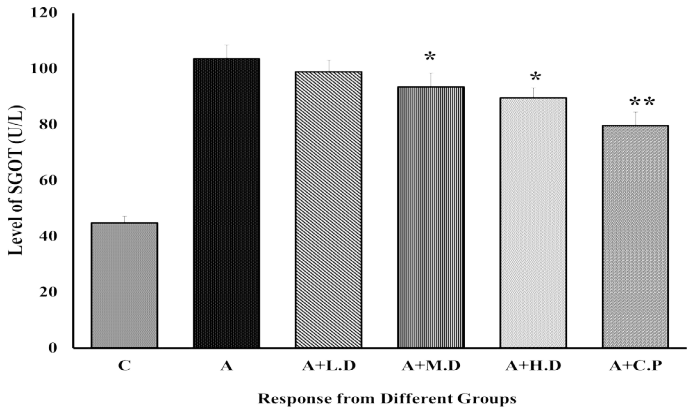

Figure 3 Camparision of SGOT level (U/L) of rats, belonged to 6 groups at day forty-two after sacrifice C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation. * Expresses the significant change,

** Expressing the High high significant change.

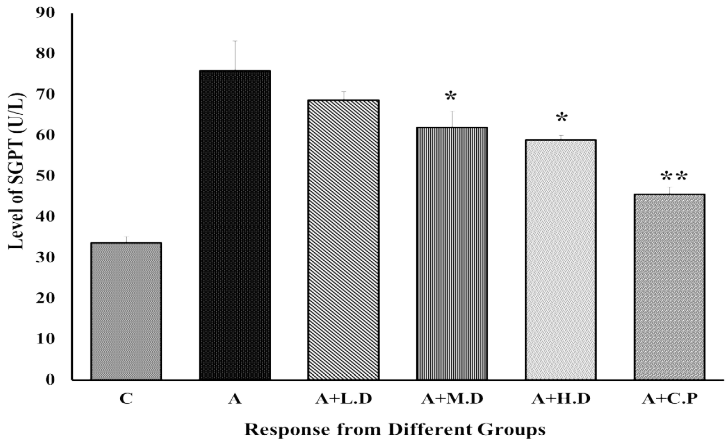

Figure 4 Camparision of SGPT level (U/L) of rats, belonged to 6 groups at day forty-two after sacrifice. C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+ H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation. * Expresses the significant change,

** Expressing the High high significant change.

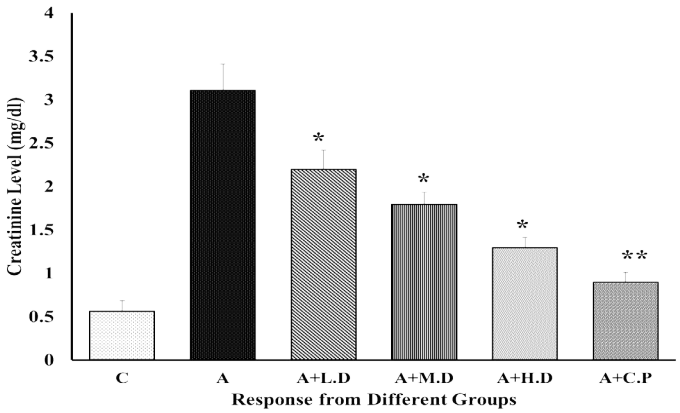

Figure 5 Camparision of Creatinine level (mg/dl) of rats, belonged to 6 groups at day forty-two after sacrifice. C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+ H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation. * Expresses the significant change,

** Expressing the High high significant change.

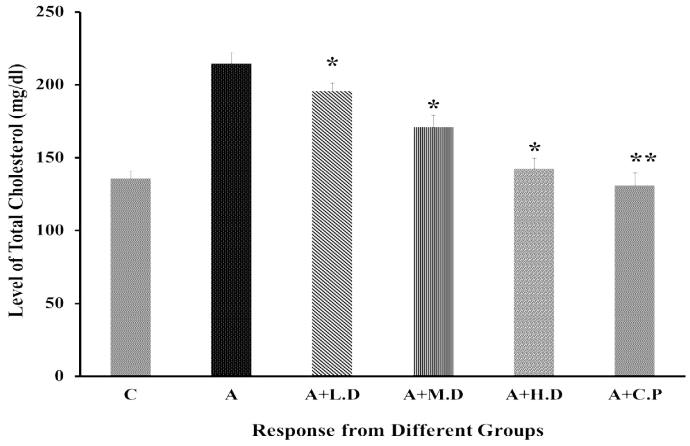

Figure 6 Camparision of Total Cholesterol Level (mg/dl) of rats, belonged to 6 groups at day forty-two after sacrifice. C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation. * Expresses the significant change, ** Expressing the High high significant change.

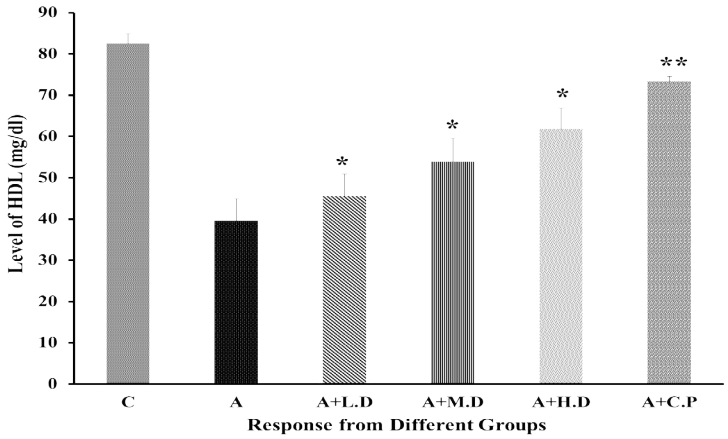

Figure 7 Camparision of HDL Level (mg/dl) of rats, belonged to 6 groups at day forty-two after sacrifice C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation. * Expresses the significant change,

** Expressing the High high significant change.

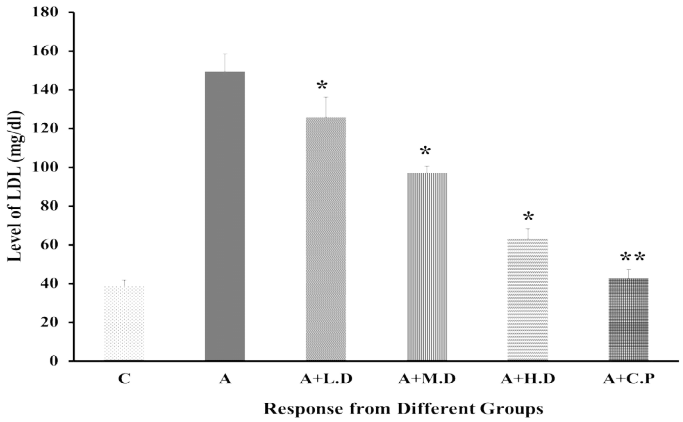

Figure 8 Comparision of LDL Level (mg/dl) of rats belong to 6 groups at day forty-two before after sacrifice. C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+ H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation. * Expresses the significant change,

** Expressing the High high significant change.

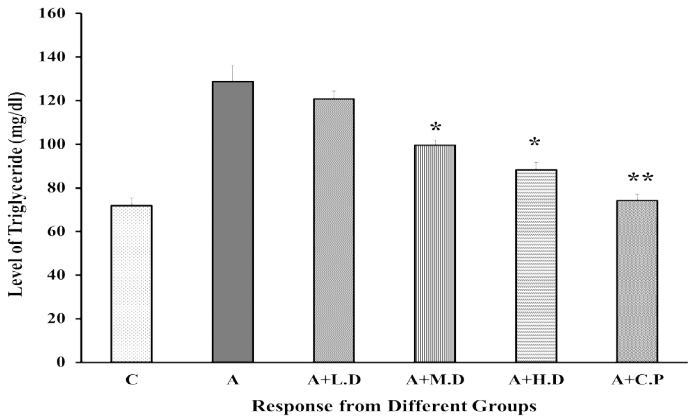

Figure 9 Camparision of Triglyceride Level (mg/dl) of rats, belonged to 6 groups at day forty-two after sacrifice. C = Control, A = Alloxan, A+L.D = Alloxan+Low Dose, A+M.D = Alloxan+Medium Dose, A+ H.D =Alloxan+High Dose, A+C.P = Alloxan+Commercial preparation. * Expresses the significant change,

** Expressing the High high significant change.

Safety Profile Study (Lipid Profile)

The below mention values regarding the level of Triglyceride level (mg/dl) of rats belonged to 6 groups are given below Figure 9.

Discussion

Body Weight Measurement

The Body weight of rats was abated in every single group when compared to negative control. Herein, the reduction of body weight was highest in diabetic control group. On the contrary, in treatment groups, with the augmentation of dose, depletion was lessened. In commercial preparation treated groups, lowest reduction in body weight was remarked.

Blood Glucose Level

Diabetic induced rats encountered an increased blood sugar level than all other groups. There was no significant reduction in the blood glucose level was seen at low dose. Whereas, the high and medium dose produce significantly decline ($p<0.05$) in blood glucose level. Furthermore, in commercial preparation, a high statistically significant fall ($p<0.01$) in blood glucose level was detected. Yet, all the groups have the potentiality to lower the elevated blood sugar level in comparison to diabetic group rats. Conversely, the blood glucose level of rats appertain to group 1 (Normal Control group) was audited to be normal.

Liver and Kidney Functioning Test

The diabetic induced group rats experienced highest level of SGOT, SGPT and Creatinine than all other groups. This may be due to deleterious effect of alloxan. On the other hand, it was inspected that the SGOT, SGPT and Creatinine levels were significantly lowered ($p<0.05$) at medium and high dose respectively. Contrarily, at low dose level null significance was observed when compared with group 2 ($p>0.05$). Furthermore, SGOT, SGPT and Creatinine levels were sharply ($p<0.01$) decreased in commercial preparation in comparison to diabetic group.

Lipid Profile

The seeds of Nigella sativa were fruitful in lowering triglycerides, LDL-C and total cholesterol in the serum at all dose levels. The reduction showed statistically significant in case of medium and high dose and in case of commercial preparation high statistical significance was observed. HDL-C level increased at all dose levels when compared to the diabetic induced group. There was no significant increase in the HDL-C level was spotted at low dose. However, a significant increase was marked at medium and high dose and high statistical significance was found in commercial preparation.

From the above discussion, it can be expressed that the commercial preparation can reduce the blood sugar level more adroitly than that of experimental lab-based preparations. Several reasons may be accountable for the lifted potentiality of commercial preparation for example: the season of seed collection, or geological area of cultivation and also can be some errors during preparations. Apart from that, the response of rat's belonged to medium and high doses resemble to be almost similar from both statistical and visual inspection. It may arise due to receptor saturation.

Conclusion

Ethanolic extract of Nigella sativa exhibit dose and source-dependent activity against diabetes. The commercial preparation imparted the best effects than that of all other groups. Most likely, several reasons for e.g; error-free lab-based preparations, geographical area of cultivation, or the season of seed collection may lie behind this deviation. Furthermore, it was also investigated that this seed extract can improve the altered pathological condition of alloxan-induced diabetic rats. From the viewpoint of the safety study, it can be terminated that the doses of ethanolic extract of Nigella sativa were not toxic to the rats and did not significantly alter the pathological state in healthy individuals. Hence, It might therefore be presumed that ethanolic extract of Nigella sativa could be effectively used as an alternative therapy in the prevention and treatment of diabetes

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