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Screening of Anti-Diabetic Potential Of Hydro-Alcoholic Seeds Extract of Nigella Sativa in Streptozotocininduced Diabetic Rats

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ABSTRACT: Herbal drugs obtained from huge number of plants/herbs were extensively utilized in the prevention, diagnosis or treatment of various human diseases since ancient era. Recently, a research suggested that Nigella sativa exhibits an anti-diabetic effect by showing marked histopathological changes in kidney and pancreas of rats. So the present was designed to evaluate the anti-diabetic potential of hydro-alcoholic seeds extract of Nigella sativa in rat model.The fresh seeds of Nigella sativa were collected from the local market and identified & authenticated by botanist at National Botanical Research Institute (CSIR), RanaPratapMarg, Lucknow with the voucher specimen no. (NBRI-SOP-202), Reference no. (NBRI/CIF/296/2012).N. sativa seeds (black seeds) were fully shade dried and extracted using hydro-alcoholic solution (1:1). Animals were procured from the animal housing facility of SPS, IFTM University Moradabad (Reg. no. 837/ac/04/CPCSEA).Adult albino rats (200-250g) of either sex were used for the screening of the effect. The Body weight determination, Oral Glucose Tolerance Test (OGTT), Blood Glucose and SGOT and SGPT levelswere evaluated for its anti-diabetic potential. In results, it significantly demonstrated the positive anti-diabetic in dose dependent manner. It clarifies its potent efficacy and safety concerns. In conclusion, it suggests identification and isolation of specific constituents being responsible for its noble pharmacological potential.

KEYWORDS: Nigella sativa, Black seeds, hepato-protective and OGTT.

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I INTRODUCTION

Herbal drugs obtained from huge number of plants/herbs were extensively utilized in the prevention, diagnosis or treatment of various human diseases since ancient era[1].Diabetes Mellitus is a group of metabolic disorders with impaired glucose metabolism that leads to an increase in blood glucose level, free radical production and increase in triglycerides and lipoproteins level with the risk of vascular and renal diseases. It has been classified as Type I, Type II and Gestational diabetes mellitus. According to World health organization (WHO) reports the number of patient is expected to be increasing progressively on daily basis. The Indian traditional system of medicine refers "science of life and longevity". The unbeaten heritage of this system is a real treasure house for both preventive and curative health care being easily available to mankind [2].*Nigella sativa* is one of the best examples of herbs which can ameliorate metabolic factors in patients suffering from diabetes mellitus. It has a numerous lifesaving properties; therefore became the highly researched plant of 21st century [3].Nigella sativa (Kalongi) belongs to Ranunculaceae family, having the bronchodilator, diuretic, immunomodulator and anti-bacterial activity etc.[4]. The awareness on this epidemic issue has led to a vast discovery of newconstituents extracted from herbal plants. There are numerous active ingredients extracted from herbal plants possessing therapeutic potentials including hypoglycemic, antioxidant action etc.Some previous researches prove about its anti-diabetic effect; extracted using different organic solvents like acetone etc. [5].Recently, a research suggested that*Nigella sativa* exhibitsan anti-diabetic effect by showing marked histopathological changes in kidney and pancreasof rats [6]. So the present was designed to evaluate the anti-diabetic potential of hydro-alcoholic seeds extract of Nigella sativa in rat model.

II MATERIAL AND METHODS

Collection andauthentication of Nigella sativa

The fresh seeds of Nigella sativa were collected from the local market and identified & authenticated by botanist at National Botanical Research Institute (CSIR), RanaPratapMarg, Lucknow with the voucher specimen no. (NBRI-SOP-202), Reference no. (NBRI/CIF/296/2012).

Extraction of black seeds[7]

The black seeds were fully shade dried for 15 days and thoroughly grinded by using electric mixer till fine power obtained. It was weighed 200g on electronic digital balance. It was extracted by Soxhlet extraction apparatus using ethanol (95%) and distilled water (1:1) solvent. The extract was filtered using whatman filter paper. Thus we obtained a clear extract of Nigella sativa seeds. Extract was kept on water bath (35-40˚C) till obtained a black powdered residue. The obtained powder was weighed (11.5g) and thus percentage yield was calculated 5.75% as below-

Percentage yield (%) = Practical yield/Theoretical yield×100
= 11.5/200×100
= 5.75

Chemicals

All the laboratory chemicals were used of standard quality. Streptozocin, SGOT & SGPT Kit were purchased from Sigma–Aldrich (Bangalore, India).Glibenclamide (anti-diabetic) was purchased from Sanofi India Ltd.

Preparation of animals

Animals were procured from the animal housing facility of SPS, IFTM University Moradabad (Reg. no. 837/ac/04/CPCSEA). Adult albino rats (200-250 g) of either sex were used in the screening of the effect. Temperature and light and dark cycle were maintained for 12:12 hours. Humidity was found approx. 40 - 60% and rats were fed with standard pellet diet and water *ad libitum*. They were fasted for 18 hours prior to experiment, with free access to water. The study was performed according to the Institutional Animal Ethics Committee [8].

Acute Oral Toxicity

The doses of Nigella sativaseeds extract were selected according to limit-test of OECD Guideline No. 423. Animals were maintained under controlled environment of 12 h dark/light cycles and temperature 22±3ºC. They had free access to food and purified water *ad libitum* for 5 days. Three rats (overnight fasting) were taken for each step. The starting dose level was chosen 5 mg/kg from four different fixed doses 5, 50, 300 & 2000 mg/kg. Animals were kept under observation after dosing once in 30 minutes, with special attention during the first 4 hr and thus daily for 14 days and body weight was taken [9].

Induction of diabetes mellitus

Adult albino rats were once injected*(i. p.)*Streptozotocin65mg/kg dissolved in 0.1 M citrate buffer and P^H 4.5.After administration, rats were observed with increased level of glucose (hyperglycemia) and glycosuria [2].

Experimental Design

Each group contains 6 animals.

Group I: Control (Diabetic): In this group all the rats were given Streptozotocin(65mg/kg),*intraperitoneally*.

Group II: Standard (Glibenclamide): In this group all the rats wereadministeredGlibenclamide(10 mg/kg) once daily, by *intra peritoneal* route for 21 days.

Group III: Test a (100mg/kg): In this group all the rats wereadministeredNigella sativa seeds extract (100mg/kg) once daily, *orally* for 21 days.

Group IV: Test b (200mg/kg): In this group all the rats were administered Nigella sativa seeds extract (200mg/kg) once daily, *orally* for 21 days.

Protocols

1. Body weight determination

Whole body weight was taken for all the group of animals on different consecutive days 5, 10, 15 and 21. Test drug treated groups were compared with control and standard[4].

2. Oral Glucose Tolerance Test (OGTT)

All the animals were orally administered glucose 2g/kg after completion of their respective administrations. Blood samples were collected from tail vein puncture at every 30, 60, 90 and 120 minutes and examined its blood glucose level with the help of glucometer (one strip technique) [10].

3. Blood Glucose level

The blood glucose level of all the treated animals were examined 1 hour after drug administered on different consecutive days 5, 10, 15 and 21. The glucometer was also employed to check the glucose level [11].

4. Serum glutamate pyruvate transaminase (SGPT) and Serum glutamate oxaloacetate transaminase (SGOT) level in liver tissue

The serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by Reitman and Frankel method. Serum 0.05 ml with 0.25 ml of substrate (aspartate and α-ketoglutarate for SGOT; alanine and α - ketoglutarate for SGPT, in phosphate buffer pH 7.4) was incubated for an hour in case of SGOT and 30 min for SGPT. A 0.25 ml of DNPH solution was added and kept for 20 min at room temperature. After incubation period, 1 ml of 0.4 N NaOH was added and absorbance was noted at 505 nm using *UV*Spectrophotometer. Values were expressed as IU/dl [12].

III. RESULT AND DISCUSSION

A. Body weight

+Table 1:Body weightdetermination

Treatment	Body Weight(Mean± SEM) 0 days5 days	10 days	15 days	21 days	
Control (Diabetic)	135.2±3.53	139.3±2.29	142.3±1.80	146.2±2.49	132.3±3.35
Standard(Glibenclamide)	161.7±3.33	157.8±2.65	164.2±2.57	176.3±2.18	181.8±1.55
Test a (100 mg/kg)	138.3±0.84	148.7±0.80	162.2±0.98	164.3±1.20	171.2±0.90
Test b (200 mg/kg)	143.8±2.24	156.7±1.62	163.8±0.79	171.3±0.80	177.3±0.71

Table 1 has depicted the positive effect of drug on body weight of animals. Test drug was given in two consecutive doses 100 and 200 mg/kg. The anti-diabetic potential of the *N. sativa* was evaluated against streptozotocin induced diabetic rats and compared with the standard drug Glibenclamide. They were kept evaluated on five consecutive days including o, 5, 10, 15 and 21 days.

The effect was found as dose dependent; maximum on higher (200 mg/kg) dose. The effect was also compared with diabetic control group. It indicates that *N. sativa* has the anti-diabetic effect but not much potent as standard.As it does not showed any oral toxicity; indicates for its safety measures.There were not many variations in the effect on the intervals of the screening (days) therefore it supports for its acute and chronic effects under safety concerns.

Pharmacological effect is seen due to presence of various constituents like alkaloids, flavonoids, tannins, glycosides, and others. It significantly increased the body weight and proved for its anti-diabetic potential.The following Fig. 1 represents the graphical interpretation of the effect on body weight.

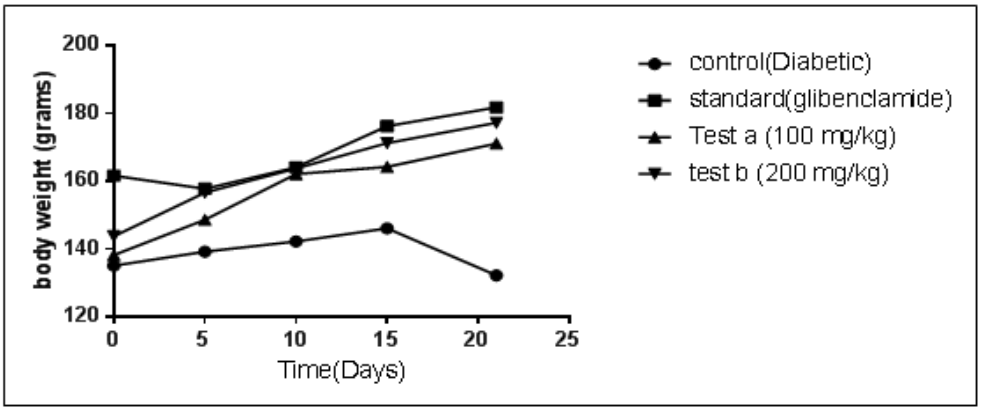

Fig. 1 Graphical interpretation of rat's body weight

B. Oral Glucose Tolerance Test (OGTT)

Table 2: Oral Glucose Tolerance Test (OGTT) Estimation

Treatment	Blood Glucose Concentration (mg/dl) 0 min	30 min	60 min	90 min	120 min
Control (Diabetic)	239.7±5.45	270.8±5.97	299.2±5.38	238.3±3.80	180.8±1.62
Standard (Glibenclamide)	172.2±2.24	232.2±3.54	201.7±7.61	171.2±3.95	129.2±3.43
Test a (100 mg/kg)	206.5±3.48	273.7±3.42	235.3±2.78	202.3±2.21	194.7±3.75
Test b (200 mg/kg)	181.5±2.04	241.7±3.65	206±5.04	185.8±2.13	137.5±2.39

The Oral Glucose tolerance test was estimated as depicted in table 2. It was estimated on several consecutive minutes including 0, 30, 60, 90 and 120 minutes. They were found most effective at 120 minutes. The effect of test drug *(N. sativa)*was compared as control, and standard group. It exhibited a significant anti-diabetic effect by controlling the increasing blood glucose concentration. The pharmacological response was

observed dose dependent because maximum effect was seen at higher dose (200mg/kg). It depicts that test drug possess the acute as well as chronic action. Presence of various constituents is responsible for its significant anti-diabetic potential comparable to standard drug. Its safety and efficacy measures are good enough to go for human use as ongoing from earlier.It was well evaluated and graphically represented as below in form of Fig. 2.

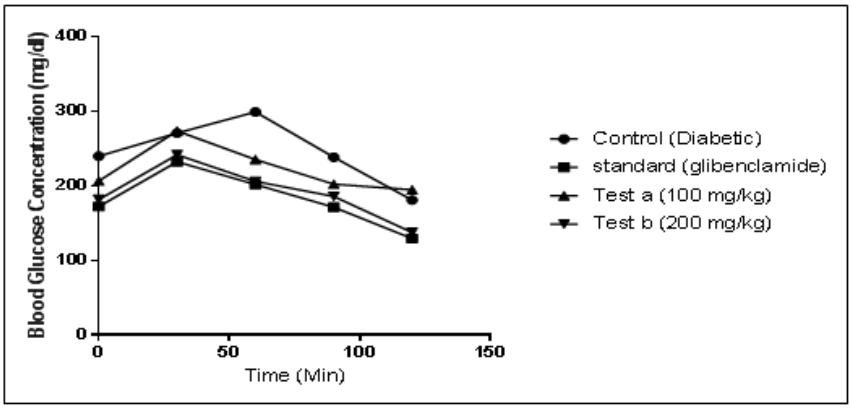

Fig. 2 Graphical interpretation of Oral Glucose Tolerance Test (OGTT)

C. Blood glucose level

Table 3: Detection of blood glucose level

Treatment	**Blood Glucose Level (mg/dl)**			
	5 days	**10 days**	**15 days**	**21 days**
Control (Diabetic)	242.2±3.25	281.5±3.99	308.5±5.88	329.7±5.20
Standard (Glibenclamide)	210.2±4.54	216.7±2.81	201.3±3.34	191.2±2.45
Test a (100 mg/kg)	189.4±5.47	201.3±3.34	200.4±3.48	180.1±3.79
Test b (200 mg/kg)	218.3±2.36	227.3±4.57	207.7±4.22	199.5±3.25

The screening of blood glucose level was depicted in table 3.It was evaluated on different consecutive days including 5, 20, 15 and 21. The effect observed was increasing progressively day by day and found effective in every measures. It demonstrated the significant level of effect as compared with control and standard drug treated groups. Presence of diverse group of constituents is responsible for its divine pharmacological effects. It exhibited a dose dependent control over the concentration of glucose in blood stream. Graphical interpretation was recorded as below in fig. 3 depicting the management of blood glucose level.

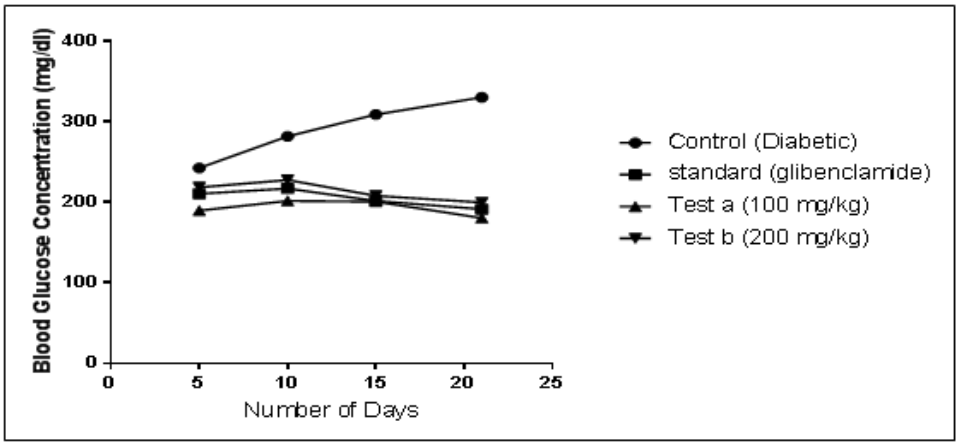

Fig. 3 Graphical interpretation of blood glucose level

D. Evaluation of SGOP and SGPT

Table 4: Detection of SGOT and SGPT levels in liver tissue

Treatment	SGOT (IU/dl)	SGPT (IU/dl)
Control (Diabetic)	165.7±3.41	143.3±5.37
Standard (Glibenclamide)	72.33±3.10	68.83±2.35
Test a (100 mg/kg)	101.3±4.21	117.3±3.77
Test b (200 mg/kg)	81.33±3.73	70.17±2.52

Table 4 depicts the estimation of SGOT and SGPT level. The level of SGOT and SGPT demonstrates the functioning of the Liver. The increased level of these two indicates the toxicity of liver that further leads to liver failure. As above table states that test drug decreased the level of SGOT and SGPT as compared to standard drug treated group.

It exhibited the dose dependent response as in others. The values were estimated in IU/dl. It proves that *N. sativa* can also be used in the liver toxicity except as anti-diabetic solely.

It was observed under laboratorial hygienic conditions and performed under given guidelines.

Fig. 4 shows its graphical interpretations.

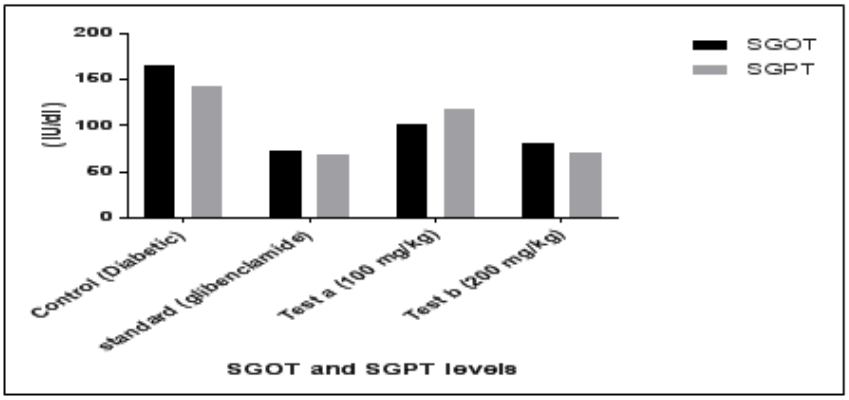

Fig. 4 Graphical interpretation of SGOT and SGPT

In our study, a significant weight loss was observed in the diabetic group while NS treated (200 mg/kg) rats exhibited significant increase in the body weight.This reported that NS markedly improved body weight gain in STZ-induced diabetic rats [13].

IV. CONCLUSION

The above experiment clarifies under results section that *N. sativa* is a potent anti-diabetic and hepato-protective potential. It demonstrated a significant potential of lowering the glucose concentration in the blood stream when compared with standard group (Glibenclamide). It proves and suggests the use in diabetes and liver toxicity in more potent form after identifying and evaluating the active constituents responsible for these noble activities.

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