Study on Anti-hyperglycaemic and Hypolipidemic activity of Nigella Sativa Seeds

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ABSTRACT

Objective: *To evaluate the anti hyperglycaemic and hypolipidemic activity of chloroform extract of seeds of Nigella sativa in alloxan induced diabetic rats.*

Materials and Methods: *Alloxon induced (150 mg/kg in normal saline i.p) diabetic rats were given chloroform extract of seeds of Nigella sativa (400mg/kg, 800mg/kg, 1200mg/kg p.o n=6) or Vehicle (Tween 80 dispersion in distilled water) or standard drug Gliclazide(10mg/kg p.o) for 28 days. The blood samples were withdrawn by tail venepuncture technique and analyzed for blood glucose level by commercial glucometer with test strips. For evaluating immediate effects, blood samples were analyzed at 4th, 6th, 8th and 24th hrs. on the first day. For sub-acute study, samples were analyzed for blood glucose on 7th, 14th, 21st and 28th days. On 28th day, after overnight fasting blood samples were withdrawn by cardiac puncture under anaesthesia (Diazepam 5mg/kg + Ketamine 40mg/kg) and lipid profile estimated.*

Results: *The chloroform extract of seeds of Nigella sativa have statistically significant immediate antihyperglycaemic effects – maximum effect after 6 hrs. of administration. On daily administration, it produced sustained fall in elevated blood glucose levels. Both the immediate and sustained effects were maximum in the dose of 800mg/kg of the extract. The extract of seeds of Nigella sativa reduced the Total cholesterol (TC), Triglycerides (TGL) and increased the High density lipoprotein cholesterol (HDL-C). Thus restores the normal lipid profile.*

Conclusion: *It is concluded that single oral administration of chloroform extract of seeds of Nigella sativa decreases the blood glucose levels. Continuous use of the extract has significant and sustained anti hyperglycaemic activity in alloxon induced diabetic rats. It also has a favorable effect on restoring the normal lipid profile.*

Key Words: *Antihyperglycemic activity, hypolipidemic activity, Alloxon, Nigella sativa*

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INTRODUCTION

Diabetes is a chronic metabolic disorder. The leading cause of non-traumatic lower extremity amputation, renal failure and blindness in working age adults. Diabetes is also a major cause of premature mortality, stroke, cerebrovascular accidents, peripheral vascular diseases, congenital malformations, perinatal mortality and disability[1]. India being a developing nation, is yet to overcome the grips of infectious diseases, need to simultaneously address the galloping non-communicable diseases like diabetes, hypertension etc[2]. Different type of oral hypoglycemic agents along with insulin are available for the treating diabetes. From ancient times natural products have been used for the treatment of non-insulin dependent diabetes mellitus and still find extensive use in traditional medicine worldwide. Seeds of Nigella sativa (NS)-(Family: Ranunculaceae)[3] has been used for medicinal purposes for centuries in Asia, Middle East and Africa. These Nigella sativa seeds (NSS) are traditionally used as carminative, diuretic, antihelminthic, galactogogue and antibacterial[4]. Tincture of these seeds are useful in indigestion, diarorhea, Dropsy, amenorrhea and dysmenorrhea. It has got antiseptic, hepatoprotective, cytotoxic, anti-inflammatory, hypolipidemic[5] and anti-diabetic activity. The objective of this study is to evaluate the anti-diabetic and hypolipidemic activity of chloroform extract of seeds of Nigella sativa in alloxon induced diabetic rats.

MATERIAL AND METHODS

Seeds of Nigella sativa was bought from a traditional herbal shop and identification and authentication of the specimen was done by Dr. T. Venkatrathina Kumar, Assistant professor in the Dept. of Pharmacognosy, Madurai medical college, Madurai. About 500gms of shade dried and coarsely powdered seeds of NS was defatted with petroleum ether (AR grade 60-80) by cold maceration process for 72 hrs. The defatted marc was extracted with chloroform. The extract so obtained was oily in nature and it was emulsified with Tween 80 with the help of probe type of ultra-sonicator to make the product suitable for oral administration. Final concentration of the extract was 140mg/ml volume of emulsion.

Phytochemical screening and Thin Layer Chromatography: In order to determine the presence of alkaloids, flavanoid glycosides, saponins, sterols, volatile oils like Thymoquinones, phytochemical study and thin layer chromatography (Table 1 & 2) with the seed extract was performed.

Animals: Healthy inbred male albino rats (Central animal house, Institute of Pharmacology, Madurai medical college, Madurai) weighing between 200-225 gms were used for the study. Animals were fed with pellet diet (Poultry research station, Tamil Nadu Veterinary and Animal Sciences University, Chennai-35) and tap water ad libitum. Animal study was performed in the Institute of Pharmacology, Madurai Medical College, Madurai after approval from Institutional Animal Ethical Committee (IAEC-Ref No11519/E1/4/2009). Animal handling was performed according to Good Laboratory Practice (GLP).

Table 1: Acute Study– Effect on Blood Glucose Levels (mg/dl)

Group	0 hr	4th hr	6th hr	8th hr	24th hr
Control (Tween 80)	450.33±54.03	463.00±54.00	472.50±52.77	479.00±52.33	482.17±53.07
Standard (Gliclazide)	438.50±62.18	268.33±50.94 ***	247.83±50.03***	330.50±40.29***	392.00±57.66*
Test 1 (chloroform extract of Nigella sativa (400 mg/kg)	445.00±65.20	346.00±62.13**	335.17±61.80**	377.50±70.20*	425.17±62.74
Test 2 (chloroform extract of Nigella sativa (800 mg/kg)	445.67±56.59	313.83±58.99**	280.67±52.30***	349.67±52.66**	404.50±55.16*
Test 3 (chloroform extract of Nigella sativa (1200 mg/kg)	473.17±59.26	331.00±66.04**	317.33±55.19***	411.17±60.35	450.50±59.41

Values are mean + SD, n=6 in each group ***P<0.001, **P<0.01, * P<0.05 (One way ANOVA)

Table 2: Sub Acute Study –Effect on Blood Glucose Levels (mg/dl)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Control (Tween 80)	450.33±54.03	470.00±49.88	493.67±48.96	514.00±51.53	528.17±49.97
Standard (Gliclazide)	438.50±62.18	331.00±52.27***	309.17±51.08***	256.33±40.11***	235.33±35.88***
Test 1 (chloroform extract of Nigella sativa (400 mg/kg)	445.00±65.20	383.67±57.84*	374.33±61.09**	361.50±63.98**	349.50±64.84***
Test 2 (chloroform extract of Nigella sativa (800 mg/kg)	445.67±56.59	348.33±43.51**	332.33±45.30***	307.69±43.78***	288.83±47.62***
Test 3 (chloroform extract of Nigella sativa (1200 mg/kg)	473.17±59.26	403.00±50.66*	390.17±54.71**	365.50±61.59**	356.33±59.12***

Values are mean + SD, n=6 in each group ***P<0.001, **P<0.01, * P<0.05 (One way ANOVA)

Table 3: Sub Acute Study – Effect on Lipid Profile (mg/dl)

Group	Total cholesterol	Triglycerides (TGL)	High density lipoproteins (HDL)
Control (Tween 80)	139.32±19.72	236.94±61.12	38.09±2.83
Test 1 (chloroform extract of Nigella sativa (400 mg/kg)	102.56±5.00**	133.28±8.01**	59.88±1.80***
Test 2 (chloroform extract of Nigella sativa (800 mg/kg)	92.06 ± 7.53***	128.83 ± 15.82**	51.21 ± 6.33***
Test 3 (chloroform extract of Nigella sativa (1200 mg/kg)	92.22 ± 2.51**	110.89 ± 8.40**	50.06 ± 2.35***

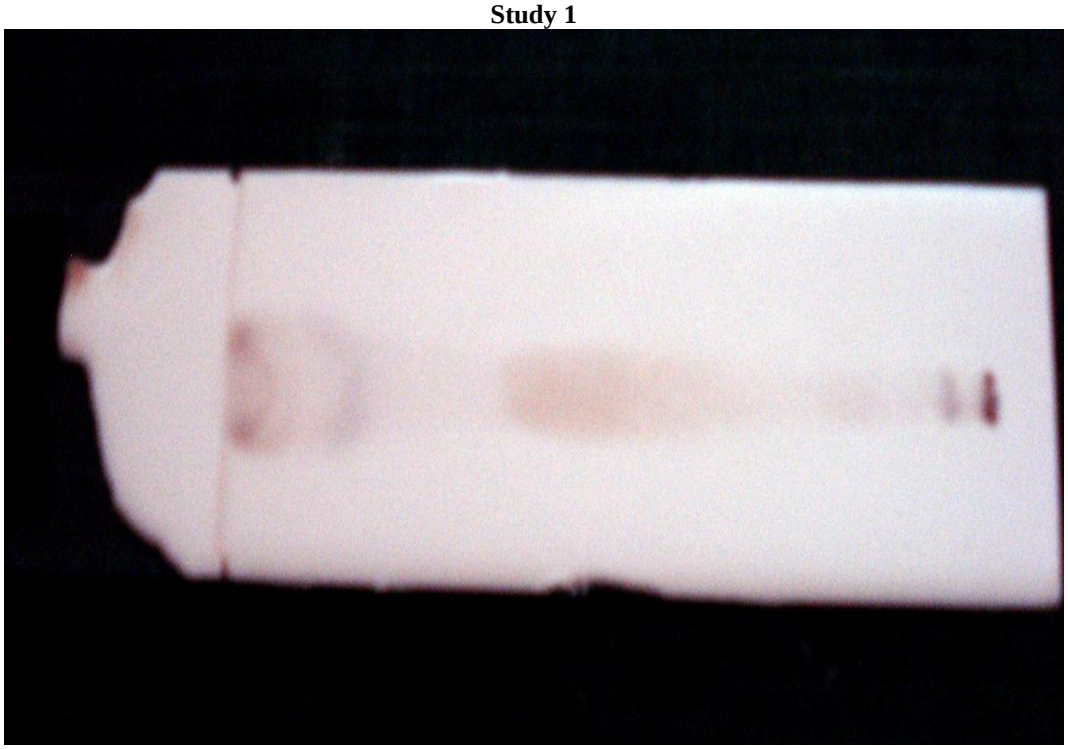
Values are mean + SD, n=6 in each group ***P<0.001, **P<0.01, * P<0.05 (One way ANOVA)

Table 4: Sub Acute Study – Effect on Body Weight (Gms)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Control (Tween 80)	215.33±6.50	207.33±7.9	198.17±6.85	190.17±6.56	187.33±5.57
Standard (Gliclazide)	213.50±7.56	221.83±7.25	223.33±7.15*	233.33±6.62***	234.83±7.57***
Test 1 (chloroform extract of Nigella sativa (400 mg/kg)	211.83±6.21	213.50±5.24	218.50±4.42	220.00±5.33*	220.83±7.36*
Test 2 (chloroform extract of Nigella sativa (800 mg/kg)	212.67±8.21	214.83±8.89	219.67±9.27	225.67±9.97*	228.00±7.90**
Test 3 (chloroform extract of Nigella sativa (1200 mg/kg)	213.33±6.44	215.00±4.98	219.50±5.99	222.17±7.68	224.33±6.77*

Values are mean + SD, n=6 in each group ***P<0.001, **P<0.01, * P<0.05 (One way ANOVA)

Study 1



Study 2
Thin Layer Chromatography

METHODOLOGY

Before inducing diabetes, normative data for fasting blood sugar(FBS), total cholesterol(TC), triglycerides(TGL), high density lipoproteins (HDL-C), and post prandial blood sugar-2 hrs. after access to food (PPBS) were estimated in randomly selected six animals from the 30 animals choosen for the study. Blood samples were taken by cardiac puncture under anaesthesia (Diazepam 5mg/kg + Ketamine 40mg/ kg).[6]

Induction of diabetes in rats: Diabetes was induced in rats with Alloxon (B.No:G204207-LOBA chemicals Pvt. Ltd.) by a single intra peritoneal injection

(150mg/kg[7] in isotonic saline[8] PH-7.4) after overnight fasting. After 72 hrs animals with FBS above 200mg/dl were selected for the study which was divided into 5 groups of 6 each. Group 1 was kept as diabetic control which received Tween 80 dispersion in distilled water. Group 2 was the standard group which was treated with Gliclazide 10mg/kg p.o daily[9] (IPCA pharma ltd-Glycinorm 80).Three test groups (T 1, T 2, T 3) received chloroform extract of Nigella sativa seeds (NSS) at doses of 400mg/kg, 800mg/kg, 1200mg/kg[10] respectively through oral route daily.

Collection of blood and estimation of blood glucose level: The blood glucose was measured by using Sugar scan (Thyrocare) glucometer with strips. Blood sample was obtained by tail venepuncture technique. The lipid profile was estimated by standard enzymatic methods using colorimeter (Dalal Photo electric Colorimeter) in the Institute of Pharmacology, MMC, Madurai.

Effect on blood glugose level: Study for acute activity involved estimation of blood at 4^{th}, 6^{th}, 8^{th} and 24^{th} hrs. after administration of vehicle, Gliclazide and different doses of chloroform extract of Nigella sativa seeds(NSS). The results tabulated and the data analysed statistically using one way anova test.

In sub-acute study– vehicle (Tween 80), Gliclazide and different doses of chloroform extract of NSS administered p.o daily for 28 days. Blood glucose was estimated on 7^{th}, 14^{th}, 21^{st} and 28^{th} day by glucometer. The results tabulated and the data analyzed statistically using one way anova test.

Effect on body weight: The weights of all the rats were recorded on days 0, 7, 14, 21, and 28. The changes in weight are tabulated and analyzed.

Effect on lipid profile: At the end of the study, after overnight fasting blood samples were withdrawn by cardiac puncture under anaesthesia (Diazepam 5mg/kg + Ketamine 40mg/kg) and lipid profile estimated. The values tabulated and analyzed. Then all the animals were euthanized with overdose of Ketamine as per CPCSEA guidelines.

RESULTS

Study on acute anti-hyperglycemic effect revealed that the single dose of different concentrations of chloroform extract of NSS reduces blood sugar levels significantly in alloxon induced diabetic rats at 4^{th}, 6^{th}, 8^{th} and 24^{th} hrs. Maximum reduction (37.02%) was seen with the dose of 800mg/kg after 6 hrs. of administration as compared with Gliclazide which produced 43.48% reduction in blood sugar level. (Table 1)

On repeated administration **(subacute study)** of vehicle (Tween 80) or Gliclazide or different doses of chloroform extract of NSS administered p.o daily for 28 days the reduction in blood sugar was highly significant with Gliclazide and all three test groups. Animals which received 800mg/kg showed sustained fall on days 14, 21 and 28. On 28^{th} day, 800mg/kg dose decreased the blood sugar level by 35.19% as compared with Gliclazide which produced 46.33% reduction.(Table 2)

Hypolipidemic effects: In alloxon induced diabetic rats dyslipedemia was observed with a rise of Total cholesterol (64mg% to 139mg%), Triglycerides (65mg% to236mg%), and fall in HDL–cholesterol (44.2mg% to 38.02mg%) in 28 days. In the test group with doses of 800mg/kg for 28 days there was significant reduction in TC ($P<0.001$), TGL ($P<0.01$) and highly significant increase in HDL ($P<0.001$). Table3.

Effect on body weight: There was a significant weight loss (13%) in vehicle treated diabetic rats whereas with standard drug Gliclazide(9.99%) and different doses of chloroform extract of NSS produced a gradual gain in body weight (7.2% with 800mg/day of the extract) respectively.(Table 4)

DISCUSSION

In view of global epidemic of obesity and diabetes – diabesity[11], there is an active resurgence of the widespread scientific interest in the medicinal plants. The chloroform extract of NSS has got a significant immediate and sustained anti-hyperglycemic effects and also restores the dyslipedemic changes.

In the acute study, at doses of 800mg/kg of the extract showed significant anti-hyperglycemic effect at 4^{th}, 6^{th}, 8^{th} and 24^{th} hrs. At doses of 400mg/kg, the anti-hyperglycemic effect was not significant at 24^{th} hr. because the dose may be inadequate. At doses of 1200mg/kg, the anti-hyperglycemic effect was not significant at 8^{th} & 24^{th} hrs. –may be due to faster elimination. In the sub-acute study, on 28^{th} day 800mg/kg of the extract decreased the blood sugar level by 35.19% as compared with Gliclazide which produced 46.33% reduction.

Alloxon, a selective β cytotoxin induces experimental diabetes by selectively damaging the insulin secreting β cells of the pancreas[12]. The possible mechanism proposed for the anti-hyperglycaemic effect of NSS may be attributed to β cell regeneration[13]. The chloroform extract of NSS also have beneficial action in up regulation of hepatic glycolytic enzymes and down regulating glyconeogenesis[14] enzymes which have the major role in diabetic hyper glycaemia. Generation of reaction species in endothelial cells is one of the detrimental effects of AGE (Advanced glycation end products) formed at an increased rate in the presence of hyperglycaemia[15]. Hyper glycaemia evoked oxidative stress plays a crucial role in the development of diabetic complications like

nephropathy, neuropathy, retinopathy and hepatopathy which are considered to be due to augmented reactive oxygen species generation[16]. The crude oil of NS and its fractions (neutral lipids, glycolipids and phospholipids) showed potent radical scavenging activity[17].Nigella sativa also mediates glugose induced insulin release[18]. It is postulated that the hypoglycaemic effect of NSS may be at least partly mediated by stimulated insulin release[19].

In type2 DM, lack of transcription of lipoprotein lipase leads to a distinct diabetic dyslipideamia characterized by high LDL, low HDL and qualitative changes in VLDL particles[20]. This will predispose to coronary heart diseases, stroke and atherosclerosis. The chloroform extract of NSS reduced the serum total cholesterol, TGL and increased the serum HDL. This restores the normal lipid profile. PUFA and constituents like alkaloids, terpinoids and sterols present in the extract may be responsible for its hypolipidaemic activity[21].

The induction of diabetes entails a significant and successive reduction of weight in rats. The maximum loss of weight (13%) reached towards the end of the experiment in control group whereas there is a gain in weight (9.99%) in standard group and (7.2%) in the test group with NSS at doses of 800mg/day. There was a gradual gain in body weight with the remaining dose of NSS also. This may be partly due to proper glycemic control and partly due to promotion of general wellbeing.

LIMITATIONS

The study has to be done in a larger sample size.

CONCLUSION

The chloroform extract of Nigella sativa seeds has anti-hyperglycemic and hypolipidemic activity in diabetic rats. It also promotes gradual weight in diabetic rats. However the exact mechanism and components responsible for the anti-hyperglycemic and hypolipidemic activity of the extract have to be scientifically explored.

BIBLIOGRAPHY

1. Michael M. Engelgau and Linda S. Geiss. The burden of Diabetes mellitus. In: Jack L, Leahy, Nathaniel G. Clark, William T. Cefalu, In: Medical management of diabetes mellitus.Marcel Dekkar,2000;1.
2. Sathyanarayana. K. Diabetes Research: Are we doing enough?. The Indian Journal of Medical Research March 2007;125(3):200-01.
3. The Ayurvedic pharmacopoeia of India. Part I. 1st ed. vol. 1, Ministry of Health and Family welfare,2001;119.
4. Indian Medicinal Plants. Volume 4, Orient Longman Ltd.,1995;2498.
5. Mai Le P, Benhaddou-Andaloussi A et al. The petroleum ether extract of Nigella sativa exerts lipid-lowering and insulin-sensitizing actions in the rat. J. Ethnopharmacol 2004; 94:251-259.
6. Diane J. Gaertner, Troy M. Hallman et al. Anaesthesia and Analgesia for Laboratory Rodents. In: Richard E. Fish, Marilyn J. Brown. Anesthesia and Analgesia in laboratory animals, 2nd ed. Elsevier; 2008. p 259.
7. Kulkarni. S. K. Handbook of Experimental Pharmacology., 2nd ed. Vallabh Prakashan;2006. p190.
8. Joy KL, Kuttan R. J. Ethnopharmacol 1990;67:143.
9. Dachicourt, N., D. Bailbe, et al. Effect of gliclazide treatment on insulin secretion and beta-cell mass in non-insulin dependent diabetic Goto-Kakisaki rats. Eur. J. Pharmacol 1998;361:243-251.
10. Zahira Houcher, Khaouther et al. Effects of Methanolic extract and commercial oil of Nigella Sativa L On blood glucose and Antioxidant capacity in Alloxan – induced diabetic rats. In. Pteridines. vol 18,2007;8-18.
11. Ashok, DB Vaidya, Rama Ashok, et al. Current status of Indigenous Drugs and Alternative Medicine in the Management of Diabetes mellitus. In: B.B. Tripathy. Text book of Diabetes Mellitus. 2nd ed. RSSDI;2008. vol 1:695.
12. Gupta. SK. Drug Screening Methods. 2nd ed. Jaypee Brothers;2009. p 590.
13. Matira Khanam and Zesmin Fauzia Dewan. Effects of the crude and the n-hexane extract of Nigella sativa Linn. (Kalaijira) upon diabetic rats. In: Bangladesh J. Pharmacol 2008;4: 17-20.
14. A.M. Mohammed, F.Z, EL Sharkawy et al. Glycemic control and therapeutic effect of Nigella Sativa and Curnuma Longa on Rats with Streptozotocin – Induced Diabetic Hepatopathy. Journal of Pharmacology and Toxicology 2009; 1-13.
15. Anirban Maitra. The Endocrine System. In: Pathologic Basis of Disease, 8th ed. Saunders Elsevier;2010. p 1138.
16. Brownlee, M., Biochemistry and molecular cell biology of diabetic complications. Nature 2001;141:813-820.
17. Hamden, H., M.A. Boujbiha., et al. Combined vitamins (C and E) and insulin improve oxidative stress and pancreatic and hepatic injury in alloxan diabetic rats. Bimed. Pharmacother. J. biopha 2008;02.001.
18. Rchid H, Chevassus H, et al. Nigella sativa seed extracts enhance glucose-induced insulin release from rat-isolated Langerhans islets. Fundam Clin Pharmacol 2004 Oct;18(5):525-9.
19. Rendell, M., The role of sulphonylurease in the management of type 2 diabetes mellitus. Drugs 2004;64:1339-1358.
20. Umesh Masharani. Diabetes Mellitus and Hypoglycemia. In: Stephen J. Mcphee. Current Medical Diagnosis and treatment, 48th ed. Mc Graw Hill Medical; 2009. p 1058.
21. Ali B H, Gerald Blunden. Pharmacological and toxicological properties of Nigella sativa. Phytotherapy Research, April 2003;299-305.