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Research Article

Nigella sativa L. seeds melanin: A new hypoglycemic agent. Comparison with insulin in alloxan-diabetic rats

Al-Tayib O.A.[1]*, El Tahir K.E[2], Idriss M.H[3], Eram K.E[4], Hassib A.M.[5]
[1]Eng. A.B. Research Chair for Growth Factors and Bone Regeneration, College of Dentistry, King Saud University, Riyadh, Saudi Arabia;
[2]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia;
[3]Department of Stem Cells, College of Medicine, King Saud University, Riyadh, Saudi Arabia;
[4]Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia;
[5]Department of Physics, College of Science, King Saud University, Riyadh, Saudi Arabia.

***Corresponding author**
Omar A. Al-Tayib
Email: altayibomar@gmail.com

Abstract: The influence of *Nigella sativa* seeds melanin and insulin was compared in alloxan (185 mg/kg/i.p.) - diabetic rats regarding the blood glucose level, blood cells and other parameters and the body weights. Treatment of the animals with melanin 50 mg/kg i.p daily for three days or with insulin 5 IU/kg i.p for the same period induced significant decreases in the blood glucose level with a maximum of (49.5%) and (47.8%) on the 3rd day of treatment, respectively. Both treatments induced significant decreases in the number of leukocytes and lymphocytes (4.2-13.7%); compared with the diabetic control animals. Diabetes alone or melanin and insulin treatment of the diabetic animals suppressed the normal increase in body weight observed in the control non-diabetic animals. On broad basis, *Nigella sativa* melanin seemed to be a potential hypoglycemic agent.
Keywords: *Nigella sativa;* melanin; alloxan; diabetic; rats

INTRODUCTION

Since the recognition of diabetes mellitus in humans and till the discovery of insulin in 1925 and even thereafter, the plant kingdom provided a major source for treatment of this disease. In fact, the last 5 decades witnessed a huge literature regarding discovery of plants that showed efficacy in treatment of this disease in both humans and animals [1-2]. In recent years, the mammalian pigment-melanin has been discovered in many plant seeds [3-4]. It has been discovered and isolated melanin from the outer black coat of the seeds and tissue culture of the plant *Nigella sativa*, botanical family Ranunculaceae in a yield of 2% in the seed outer coats [5-6]. This plant is known in the Arabian and Islamic countries as Habbat Albaraka or Shuniz and in English language as Black Cummin. Pharmacological investigations revealed a potent anti-inflammatory, anti gastric ulcerogenic and anti-oxidant and hepatoprotective activities [7]. Furthermore, it is shown to activate Toll-like receptor 4 (TLR 4) [8] and to release IL-6 [9].

Various anti-oxidants e.g plant flavonoids such as quercetin have been shown to be effective in decreasing blood glucose level in experimentally-induced diabetes[10-11]. This observation coupled with the driving need for a safer hypoglycemic agent free from the side effects of anemia, leucopenia, thrombocytopenia or lactic acidosis observed with the available hypoglycemic [12-14]. Several phytomedicine have been reported to possess anti-diabetic and hypoglycemic potentials. The technique usually requires using plant parts, e.g. seeds, roots, leaves, bark etc to prepare decoctions, syrups, extracts or tinctures for medicinal purposes. However, some phytomedicine were not yet found their relative activities such as *Nigella sativa L. seeds*; it was thought of interest to examine the effects of a water- soluble melanin extracted from the outer black coats of the seeds of the plant *N. sativa* seeds melanin (NSM) for the first to determine the hypoglycemic effects and compare with insulin on alloxan- induced diabetes in rats and to compare its actions with those of insulin.

MATERIALS AND METHODS

Animals

Male Wistar rats (190 ± 10 g body weight) were obtained from the Experimental Animals Care Center, College of Pharmacy, King Saud University, Kingdom of Saudi Arabia. They were maintained in normal rat chow and water *ad libitum*. They were housed in cages in a room at temperature of 22 ± 2 °C

and a relative humidity of 55 ± 5%. The light/dark cycle was 12/12 h. The protocol of the experiments was approved by the College Ethical Committee. All animals were weighed at the start and the end of the treatment.

Induction of alloxan diabetes:

Diabetes was induced in the rats by a single injection of alloxan (Sigma-Aldrich, Germany) at a dose of 185 mg/kg (I.P.). Alloxan was dissolved in a normal saline solution before injecting. All diabetic animals were used after 3 days (blood glucose level > 250 mg %).

Determination of the blood glucose level:

The blood glucose level to confirm diabetes in the different treatment groups was determined after injection by measuring the tail vein blood glucose level with an Accu-Check Sensor Comfert glucometer and test strips (Roche, Germany).

Determination of the hematological parameters:

Total blood cells counts (CBC) and the other hematological parameters were determined in blood with (EDTA) anti-coagulant using electronic counter: (Vetscan HMII, Abaxis Veterinary Diagnostics, Northern California, USA).

Experimental protocol:

Initially, pilot experiments were performed to find out the effectiveness of melanin in reducing blood glucose levels. Melanin was tested in a dose up to 200 mg/kg (i.p). A sub maximal dose of 50 mg/kg i.p was then chosen to compare it with insulin at a dose of (0.8 IU/kg daily).

After induction of diabetes the rats were divided randomly into 3 groups (N= 5 animals per group). The first group was not treated and is labeled Diabetic Control group. The second group was injected daily for 3 consecutive days with melanin (50 mg/kg i.p.) and the third group was injected similarly with insulin (0.8 IU/kg i.p.). A fourth group (N= 5 animals per group) of non-diabetic animals was also included. The control non-diabetic group and the control diabetic group were injected with distilled water daily as above in a volume of 1.6 ml/kg i.p. every day. The blood glucose level was determined just before the injection and 2 hours after. This was done during the 3 days treatment. For the determination of the hematological parameters, blood was collected on the third day from all of the groups 2 hours after the last doses.

Source of Melanin:

The water soluble melanin used in these studies was supplied as a gift by its discoverer and manufacturer Professor Adil Hassib, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia. Melanin was dissolved in distilled water to provide a final concentration of (31.25 mg/ml).

Statistical analysis:

The data were expressed as mean ± s.e.mean. Significant differences between the controls and the treated groups were calculated using ANOVA with $p<0.05$ taken as significant.

RESULTS

Effect of alloxan:

Treatment of the rats with alloxan increased the blood glucose level from 183.2± 5.1 to 507.4± 6.3 mg% 3 days after treatment, an increase of 267 %. On the last day of treatment, the blood glucose level in the diabetic control group reached 445.6 ± 12.9 mg% - an increase of 222.4 % compared with the non-diabetic control group (Tables 1 and 2).

Effect of melanin:

Treatment of the diabetic rats with melanin 50 mg/kg i.p daily for 3 days significantly decreased the blood glucose level in a duration-dependent manner (Table 1). The percentage decreases on days 1, 2 and 3 following treatment were 14.7, 42.5 and 49.5 %, respectively, ($P<0.05$, N= 5).

Effect of insulin:

Treatment of the diabetic rats with insulin (0.8 IU/kg i.p.) daily for 3 days significantly decreased the blood glucose level (Table 2). The percentage decreases on days 1, 2 and 3 were 50.6, 66.2 and 47.8 %, respectively, ($P<0.05$, N= 5).

Effect of alloxan treatment on blood parameters:

Treatment of the rats with alloxan and determination of the hematological parameters six days later revealed only a significant increase in the number of monocytes by 50 % and granulocytes 41.4% ($P<0.05$, N= 5). There were non-significant decreases in the number of the WBCs (4%), lymphocytes (28%) and RBCs (6.8%) compared with the non-diabetic control group (Table3).

Effect of melanin on blood parameters:

Treatment of the diabetic rats for 3 days with melanin induced non-significant decreases in the numbers of RBCs (11%), WBCs (4.2 %), lymphocytes (28 %) and the granulocytes (20 %) compared with the control diabetic rats ($P>0.05$, N= 5) (Table 3).

Effect of insulin on blood parameters:

Treatment of the diabetic rats with insulin for 3 days induced non-significant decreases in the numbers of the WBCs (13.7 %) and the lymphocytes (20%) compared with the control diabetic rats ($P>0.05$, N= 5) (Table 3) but induced a significant decrease (29.7 %) compared with the control diabetic rats ($P<0.05$, N= 5).

Effect of the treatment on body weight:

In the control non-diabetic rats and control diabetic rats, a clear increase in the body weight was noted it was equivalent to 6.07% and 2.90%,

respectively. However, a decrease of (7.44 to 15.58%) was noted in the diabetic rats treated with insulin and melanin-treated animals.

Table-1: Effects of Melanin on Blood glucose level in alloxan diabetic rats:

Day of Treatment	Blood Glucose mg%		Diabetic Melanin Group Blood Glucose Level mg%		
	Non-diabetic control group	Diabetic control group	Before Treatment	2h After Treatment	% Decrease
1	132.4±4.1	395.4±11.3	434.4±6	370.2±9.3	14.7
2	128.2±6.2	464.4±14.6	356±8	204.6±8.3	42.5
3	138.2±3.9	507.4±6.9	445.6±11	225 ±9.7	49.5

*P< 0.05 (N= 5) compared with the diabetic level before treatment.
**P< 0.05 (N=5) compared with the level in non-diabetic control.

Table-2: Effect of insulin on blood glucose level in alloxan diabetic rats:

Day of Treatment	Blood Glucose mg%		Diabetic Insulin Group Blood Glucose Level mg%		
	Non-diabetic control group	Diabetic control group	Before Treatment	2h After Treatment	% Decrease
1	132.4±4.1	395.4±11.3	429.6±11.9	212±5.9	50.6
2	128.2±6.2	464.4±14.6	416.4±12.3	140.6±10.3	66.2
3	138.2±3.9	507.4±6.9	463.6±8.9	241.6±12	47.8

*P< 0.05 (N= 5) compared with the diabetic level before treatment.
**P< 0.05 (N=5) compared with the level in non-diabetic control.

Table-3: Hematological values of the various groups:

NO.	Treatment groups	Parameters									
		WBC (10^9/µl)	LYM (10^9/µl)	MON (10^9/µl)	GRA (10^9/µl)	RBCS (10^6/µl)	HB (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
1	**Non-diabetic control**	10.09	6.51	0.36	3.23	8.28	14.9	45.54	55.2	18.0	32.68
2	**Diabetic control**	9.16	4.69	0.54*[a]	4.57*[a]	7.71	15.2	45.46	60.4	20.4	33.9
3	**Diabetics + Melanin (50 mg)**	8.77	3.37	1.67	3.65	7.1	14.7	42.64	57.3	20.7	34.4
4	**Diabetics + Insulin**	7.90	3.72	1.47	3.21*[b]	6.85	14.2	40.2	58.8	20.7	35.37

*[a] P< 0.05, (N= 5) compared with non diabetic control.
*[b] P< 0.05, (N=5) compared with the diabetics.

DISCUSSION

The results of this study clearly demonstrated the ability of *Nigella sativa* seeds melanin in a dose of 50 mg/kg to induce significant hypoglycemia in alloxan-diabetic rats to magnitude similar to that observed for insulin. However, unlike insulin it did not suppress the number of the granulocytes- the immune cells. The actual mechanism of melanin induced hypoglycemia was not persued in this study but may be related to its antioxidant and free radicals scavenging activity [15-17]. In this connection, it is pertinent to recall that other natural antioxidants and free radical scavengers such as the flavonoid quercetin induced hypoglycemia in both of alloxan-and streptozotocin-induced diabetic rats [10,11, 18].

Another plausible mechanism for melanin-induced hypoglycemia may be through its ability to induce some regeneration of the pancreatic β-cells of the islets of the Langerhans damaged by streptozotocin. Such proposed action may be due to melanin- induced release of IL6 [9]. Partial regeneration of β-cells can enhance endogenous insulin release resulting in reduction of blood glucose level. The involvement of IL6 in regeneration of β-cells has been described before[19]. Generally, a protective role of IL6 against cells, apoptosis has been previously noted [20]. Indeed, a melanin- IL6-induced regeneration of β-cells may explain the very clear hypoglycemia (> 40%) observed on the second day of melanin treatment (Table 1). On a broad basis, the discovery of melanin as a potential hypoglycemic agent without decreasing the number of the granulocytes, may pave the way for addition of a

new armament in the treatment of diabetes mellitus- a disease that is associated with serious slow complications that incorporate retinopathy, neuropathy, nephropathy, atherosclerosis, ketoacidosis and hyperglycemic hyperosmolar non-ketotic coma[21].

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