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ANTI-INFLAMMATORY EFFECTS OF *NIGELLA SATIVA* L. MELANIN

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ABSTRACT

Herbal melanin, obtained from *Nigella sativa* L., has been studied for anti-inflammatory effects against formalin-induced rat-paw oedema. Topical treatment with 2.5% melanin solution has significantly inhibited the swelling (compared to untreated induced-oedema swelling) to 75.4% after 1hr, 81.1% after 2hr and 85.6% after 3hr. Treatment with 1% hydrocortisone solution reduced the induced-oedema swelling after 1, 2 and 3hr to 32.7%, % 63.1 and % 71.9% respectively. The melanin treatment has shown a rapid and effective reduction of the induced-oedema.

KEYWORDS: *Nigella sativa* L.; Herbal melanin; hydrocortisone; paw oedema.

1. INTRODUCTION

Nigella sativa L. (NS) is a herbaceous plant of the Ranunculaceae family that has been used in the Middle and Far East as a traditional medicine for a large number of ailments, including inflammatory diseases.[1] The plant seeds have been reported to contain essential oil, fixed oil, saponins, alkaloids, flavonoids and polyphenolic compounds.[1] Ghannadi *et al.* (2005) reported that the phenolic extracts of NS show considerable analgesic and anti-inflammatory pharmacological activities.[2] We have, recently, shown an abundant existence of herbal melanin (HM) in the seed coats of this plant and reported a general characterization of it.[3,4]

Melanins are brown-black natural pigments that are widely produced by animals, plants and microorganisms and have previously been reported as anti-inflammatory agents.[5,6] Melanins comprise free radicals as a permanent fraction of their chemical structure. These stable free radicals may easily be detected by electron spin resonance (ESR) at room temperature.[7] In connection with this property they also exhibit a strong antioxidant property that is related to their photo-protective and chemo-protective roles in living organisms.[8,4] The anti-inflammatory effects of *Nigella sativa* melanin, prior to this study, have not been investigated. We report in this study the anti-inflammatory effect of topical HM on formalin-induced rat-paw oedema.

2. MATERIALS AND METHODS

2.1. Preparation of HM solution

The seeds of *Nigella sativa* L. (Black cumin), were purchased from Riyadh, Saudi Arabia market and stored at room temperature. The extraction of melanin from the seed coats of Nigella sativa L. and characterization of melanin have been carried as described before by El-Obeid *et al.*[3] 2.5% melanin solution was prepared as in El-Obeid *et al.* (2006).[3]

2.2. EXPERIMENTAL ANIMALS

Male Wistar rats (250 g body weight), provided by the Experimental Animals Care Centre, College of Pharmacy, King Saud University, Riyadh, have been used in this study. The animals were divided into groups (N = 6 animals per group). The distribution of animals in the different groups was randomized. The animals were maintained at 22 ± 1^{o}C on a 12h light dark cycle and given rat chow and water *ad libitum*. All the experimental protocols were approved by the Animal Care Committee, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2.3. Induction and measurement of rat-paw oedema by formalin injection

Male Wistar rats were acclimatized in the laboratory in grilled- bottom cage for 3 hours one day before the experiment. The restraining procedure consisted of fastening one hind limb of the animal at the hip site with a thread to the perforated bottom of the cage to allow the limb to protrude freely while the animal stayed in a fixed position. The cage containing the animal was elevated from the top of the working bench to allow the placement of a 10-ml beaker below the cage directly below the hanging limb. Oedema was induced in the rats' hind paws via the intraplantar injection of 0.2 ml of 10% formalin in water into each paw. Before injection of formalin, the paw volume (in ml) was measured using a 0.45% saline-

displacement plethysmometer (Ugo Basil Switzerland). Paw volume was then measured every hour for 3 hours. The volume of the oedema induced was calculated as the volume difference (in ml) between the pre-injection paw volume and that observed at any time point of measurement.

2.4. HM and Hydrocortisone treatment

To test the influence of melanin on the induced inflammation the following procedure was used. Five ml of 2.5% aqueous melanin were placed in a 10-ml beaker. The hind paw to be injected with formalin was then immersed into the melanin solution-by adjusting the level of the beaker on the top of the working bench-for one hour prior to the intraplantar injection of formalin. Following injection of formalin, the immersion of the injected paw into the melanin solution was continued for further three hours. The paw volume was measured every hour and the next volume of oedema was calculated as indicated above. Two groups of animals were used (N=6 animals per group), one was used as a control and the other as a test group control paws were immersed in the vehicle for melanin. The percentage effectiveness of melanin in inhibiting the induced inflammation was then calculated with reference to the control oedema. The effect of the standard anti-inflammatory drug hydrocortisone against formalin-induced rat-paw oedema was done in parallel. 1% hydrocortisone in a mixture of alcohol and propylene glycol (2:1), were placed in a 10-ml beaker and the hind paw s of rats (n=6) were immersed into the solution for 3 hours and measurements were taken same as for HM as shown above.

2.5. Statistical analysis

Statistical means were evaluated in order to determine the anti-inflammatory effect. All data were expressed as means with standard errors. Significance differences between the groups were made by the use of the student's "t" test for unpaired data. A p value of < 0.05 was considered significant. Table 1 shows the cumulative results of the mean values of the oedema induction experiments for all experiments ± standard error.

3. RESULTS AND DISCUSSION

In this study oedema has been induced in Wister rat by formalin injection in rats' paws. Intraplantar injection of formalin resulted in a time dependent increase in oedema formation reaching a maximum increase in 3 hours following the injection where the maximum volume of induced oedema was 1.32 ml. (Table 1). Treatment of the hind paws via immersion in melanin solution before and following injection of formalin significantly inhibited the

induced inflammation (Oedema) at 1h, 2h and 3h. ($P<0.05$, N= 6) (Table 1., Fig.1.). Treatment of the paws locally with 1% hydrocortisone also significantly inhibited the induced inflammation. Table 2. shows the percentage of reduction of the formalin-induced swelling in hydrocortisone-treated (1%), melanin-treated (2.5%) and untreated control rats after 1,2 and 3 hours respectively. The table shows clearly that melanin acts rapidly and more effectively than the cortisone solution against the induced paw-oedema inflammation.

The results, above, indicate that HM extracted from *Nigella sativa* L. has a strong anti-inflammatory action. Topical application of HM produces significant anti-inflammatory effects. Our results show that HM is more effective than the anti-inflammatory drug hydrocortisone.

Oedema is a swelling that occurs as a general response of the body to injury or inflammation. Among the factors involved in inflammation are reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as nitric oxide (NO).[9] The effectiveness of melanin in suppressing the induced inflammation may be due to its potential ability of inhibiting the synthesis or release of the receptors of various mediators that trigger this acute type of inflammation such as prostaglandins, Platelet-Activating Factor (PAF), Nitric Oxide (NO) or various O2 radicals such as the super oxide anion, hydroxyl radical or the peroxyl radical and probably histamine and kinins.[10,11] Since melanin is known to inhibit the production of oxygen radicals and its roles as an antioxidant and free radical scavenger have been reported[12,13], it is pertinent to suggest that the observed melanin-induced anti-inflammatory effect is related to its anti-oxidant and free radicals scavenging actions. Our results agree with the recent results reported by Xu *et al.* 2012 who demonstrated the anti-inflammatory effects of hydrogen saline, a known free radical scavenger, on carrageenan induced paw oedema.[14]

Previous studies using other melanins, revealed its inherent property in suppressing experimentally-induced inflammations. This has been demonstrated for the marine animal the squid (*Ommastrephes bartrami*) melanin[5] and the plant grape melanin.[6] Besides, our previously reported electron spin resonance (ESR) studies[3,4] have revealed a substantial occurrence of melanin in the seed coats of this well known and widely used medicinal and culinary herb, *Nigella sativa*. Melanin represents around 15% the seed coat alone; amounting to around 2.5% of the total mass of the seed. Some authors have demonstrated anti-inflammatory effects for *Nigella sativa* L total extracts or its constituents such as thymoquinone and fixed seed oil.[15] However, those studies did not take note of the rich

presence of melanin in this plant. Considering those results, this study reveals for the first time the anti-inflammatory effect of the herbal melanin of *Nigella sativa* and supports the other studies reported for melanins from animal and other sources and suggests the use of *Nigella sativa* as a rich herbal-melanin source in future approaches for prevention and treatment of inflammatory diseases.

Table 1. Effect of melanin on formalin induced paw oedema.

Treatment group	Oedema Volume (in ml), after:		
	1 hr	2 hr	3hr
Control	1.1±0.01*	1.22±0.01*	1.32±0.02*
Melanin Treated (2.5% solution)	0.27*±0.04*	0.23*±0.01*	0.19*±0.01*
Hydrocortisone (1% solution)	0.74±0.05*	0.45±0.03*	0.37±0.05*

*P<0.05, N = 6, compared with the control.

Table 2. Reduction of formalin-induced swelling as a percentage of control (no treatment) by hydrocortisone (1% solution) and for melanin (2.5% solution) treatment in 1-2-3 hours.

	Reduction of swelling %	
Time (hours)	Hydrocortisone (1% solution)	Melanin (2.5% solution)
1	32.7 %	75.4 %
2	63.1 %	81.1 %
3	71.9 %	85.6 %

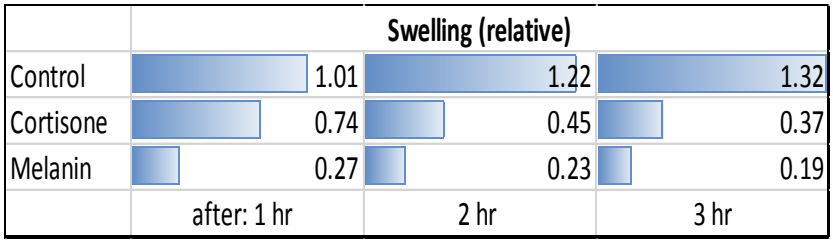

Fig 1. Rat paw formalin-induced oedema volume (ml) in 1, 2 and 3 hours after treatment with Hydrocortisone (1% solution) or with Melanin (2.5% solution) as compared to control (no treatment).

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REFERENCES

1. Salem ML. Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. Int Immunopharmacol., 2005; 5(13-14): 1749-70.
2. Ghannadi A, Hajhashemi V, Jafarabadi H. An investigation of the analgesic and anti-inflammatory effects of Nigella sativa seed polyphenols. J Med Food., 2005; 8(4): 488–93.
3. El-Obeid A, Al-Harbi S, Al-Jomah N, Hassib A. Herbal melanin modulates tumor necrosis factor alpha (TNF-a), interleulin 6(IL-6) and vascular endothelial growth (VEGF) production. Phytomedicine., 2006; 13: 324-33.
4. Hassib A. Khartoum, Sudan 1998; Patent No. 451.
5. Mimura T, Itoh S, Tsujikawa K, Nakajima H, Satake M, Kohama Y, Okabe M. Studies on biological activities of melanin from marine animals. V. Anti-inflammatory activity of low-molecular-weight melanoprotein from squid (Fr. SM II). Chem Pharm Bull (Tokyo)., 1987; 35(3): 1144-50.
6. Avramidis N, Kourounakis A, Hadjipetrou L, Senchuk V. Anti-inflammatory and immunomodulating properties of grape melanin. Inhibitory effects on paw edema and adjuvant induced disease. Arzneimittelforschung., 1998; 48(7): 764-71.
7. Mason HS, Ingram DJ, Allen B. Free Radical properity of melanin. Archives of Biochemistry and Biophysics 1960; 86: 225-30.
8. Korytowski W, Sarna T. Antioxidant effect of neuromelanin. The mechanism of inhibitory effect on lipid peroxidation. Archives of Biochemistry and Biophysics, 1995; 319: 142-48.
9. Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT. Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema. Eur J Pharmacol., 1996; 303(3): 217-20.
10. Vane JR, Mitchel JA, Appleton C, Tomlinson A. Bishop-Bailey D, Croxtall J, Willoughby DA. Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. Proc. Natl. Acad. Sci. USA, 1994; 91(6): 2046-50.
11. Miller MU, Grisham MB. Nitric oxide as a mediator of inflammation? You had better believe it. Mediators Inflammation, 1995; 4: 387-96.
12. Kumar CG, Mongolla P, Pombala S, Kamle A, Joseph J. Physicochemical characterization and antioxidant activity of melanin from a novel strain of Aspergillus bridgeri. ICTF-Lett Appl Microbiol., 2011; 53(3): 350-8.

13. Tada M, Kohno M, Niwan Y. Scavenging or Quenching Effect of Melanin on Superoxide Anion and Singlet Oxygen Journal of Clinical Biochemistry and Nutrition., 2010; 46(3): 224-8.
14. Xu Z, Zhou J, Cai J, Zhu Z, Sun X, Jiang C. J. Anti-inflammation effects of hydrogen saline in LPS activated macrophages and carrageenan induced paw oedema. J Inflamm (Lond)., 2012; 9: 2. doi: 10.1186/1476-9255-9-2.
15. Chehl N, Chipitsyna G, Gong Q, Yeo CJ, Arafat HA. Anti-inflammatory effects of the Nigella sativa seed extract, thymoquinone, in pancreatic cancer cells. HPB (Oxford)., 2009; 11(5): 373-81.