The Effect of Black Cumin Extract Cream (Nigella Sativa) On Melanin Pigment Level In Rats (Rattus Norvegicus) Exposed With Ultraviolet-B rays

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ABSTRACT
Exposure to ultraviolet (UV) radiation from the sun may cause photo aging which is characterized by uneven pigmentation and brown spots. Melanin acts to protect the skin against DNA damage due to UV radiation. Sunscreen is a cosmetic preparation that can reflect sunlight, especially in areas with UV wave emulsions. This study aims to determine the effect of black cumin extract cream on the amount of melanin pigment in rat skin exposed to ultraviolet-B rays. An experimental post-test only control group study was conducted from October 2019 - February 2020. A total of 30 rats were divided into 6 groups, namely the KN group (without treatment), P1 (basic cream), P2 (black cumin extract cream 0.25%), P3 (0.5% cream), P4 (0.75% cream) and KP (Parasol sunscreen cream). Rats in group P4 showed significantly lower amounts of melanin (41.5 ± 10.4) than other groups (KN 41.5, P1 86.0, P2 84.7 and P3 78.7; p <0.05). However, the least amount of melanin pigment was found in the KP group (10.0 ± 1.82). It can be concluded that the topical black cumin extract cream can topically reduce the amount of melanin pigment.

INTRODUCTION
Skin ageing is a complex biological process that is affected by chronic light exposure, pollution, ionizing radiation. Ultraviolet (UV) radiation from sunlight in the long term can cause premature photo aging, characterized by the appearance of changes in pigmentation and brown spots on the skin (Fisher, 2005). Melanin has an essential role in protecting the skin from UV radiation, and melanin levels are inversely correlated with DNA damage in human skin from different racial/ethnic groups (Yamaguchi et al., 2008).

Avoiding direct exposure to sunlight and consistently applying sunscreen are efforts to protect skin from UV radiation. Sunscreen is a cosmetic preparation that is used to reflect or actively absorb sunlight, especially in areas with UV and infrared wave emulsions, to prevent skin disorders caused by ultraviolet rays. Research on sunscreen has been done before with a variety of extracts such as strawberry leaf ethanol extract (Widyastuti et al., 2016), tea leaf extract (Kaur and Saraf, 2011) and dragon fruit peel extract (Daud et al., 2018). Nigella sativa, or often known as black cumin, is one of the plants with high antioxidant activity. This study aims to determine the effect of giving black cumin cream extract (Nigella Sativa) on the amount of melanin pigment in the skin tissue of male Wistar rats (Rattus norvegicus) exposed to ultraviolet-B rays.

MATERIALS AND METHODS
The Making of Black cumin Extract
Extraction was carried out by maceration by taking 1 kg of dried Simplicia powder into the vessel...
and adding 96% ethanol as much as 75 parts of 96% ethanol (7.5 litres) solvent, covered and left for five days. The maceration is then filtered and then rinsed, and the pulp is mixed with 25 parts of 96% (2.5 litres) ethanol solvent until 100 parts are obtained, then observed for two days until the macerate is obtained. The maserate is evaporated with an evaporator at a temperature of no more than 45ºC, then evaporated to achieve a thick extract.

**Cream Formulation**

The cream base formulation is a mixture consisting of 20 gram stearic acid, 1 gram Triethanolamine, 0.1 gram Nipagin, 8 gram Glycerin, 0.5 ml cetyl alcohol, 0.2 gram Sodium Hydroxide, Aquadest ad 100 ml, and Perfume 1-3 drops. The concentration of black cumin extract used in the preparation of these cream preparations was 0.25% each; 0.50% and 0.75%. The oil phase is mixed in a porcelain container and melted in a water bath at 70 ºC. The water phase is mixed into hot water that has been measured at a temperature of 70 ºC until dissolved. The oil phase is put into the mortar, and the water phase is added, crushed to form a creamy mass. In another mortar, black cumin extract is added, then added to a crushed cream until homogeneous.

**Methods**

This research is an experimental study, using a post-test only control group research design. The study was conducted from October 2019 - February 2020, starting with the determination of black cumin (Nigella sativa), extracting and making cream preparations. Before turning it on to experimental animals, the cream is first tested for homogeneity, pH testing, and the type of emulsion.

A total of 30 male rats (Rattus norvegicus) Wistar strain aged 2.5-3 months with body weight 150-180 grams and did not suffer pain were divided into six groups, KN group (without treatment), P1 (basic cream), P2 (P2 black cumin extract cream 0.25%), P3 (0.5% cream), P4 (0.75% cream) and KP (Parasol sunscreen cream). Examination of the amount of melanin pigment and histopathological examination of skin tissue after UV-B exposure. Statistical analysis was performed with the One-way ANOVA test with SPSS software.

**RESULTS**

**Cream Composition**

Before being applied to experimental animals, homogeneity tests were first performed, which showed that there were no coarse grains on glass objects, and thus all cream preparations were declared homogeneous. The resulting cream emulsion was in the form of m/a with pH values ranging from 5.8 - 6.7. The results of the phytochemical test are listed in Table 1.

**Effect of Black Cumin Extract Cream on the Amount of Melanin Pigment**

Black cumin extract cream is expected to act as a sunscreen to reduce the absorption of UV-B rays on the skin. The findings of this study can be seen in Table 2. It shows that there was a significant difference in the amount of melanin pigment after UV-B exposure in the six treatment groups. The mice in the KP group (which received the Parasol cream) showed the least amount of melanin pigment, which was 10.0 ± 1.823 (p <0.001). As for the intervention group, P4 mice (cream 0.75%) showed lower amounts of melanin pigment compared to groups of P1, P2 and P3 mice. Then a Post-Hoc analysis is performed to see the differences between each group, as shown in Figure 1.

Figure 1 shows that there was no significant difference in the number of melanin in the KN group compared to P1, P2 or P3 (p> 0.05). However, when compared with P4, a significant difference was found (p <0.01). This means that black cumin extract cream, which is proven effective in reducing the amount of melanin pigment is at a concentration of 0.75%, and this effect is not found in creams with lower concentration. However, it turns out that the amount of melanin pigment is still less found in the KP group (Parasol sunscreen), which shows that Parasol cream is always superior to sunscreen.

**Effect of Black Cumin Extract Cream on Skin Histopathology**

The study continued by looking at the direct effect of giving black cumin extract cream on the histopathological picture of the skin. Figure 2 shows melanin pigment granules found in the KP group (getting
Figure 2: Histological features of P4 rat skin (figure A) and KP (picture B). Red arrows indicate melanin pigment granules

Table 1: Phytochemical test of black cumin extract cream preparation

| Parameter          | Results |
|--------------------|---------|
| Alkaloid           | Positive|
| Tanin              | Positive|
| Saponin            | Positive|
| Triterpen / Steroid| Negative|
| Flavonoid          | Positive|
| Glikosida          | Positive|

Table 2: Effect of black cumin extract cream on the amount of melanin pigment

| Group     | Amount of Melanin Mean ± SD | p value |
|-----------|----------------------------|---------|
| Group 1 (KN) | 84.0 ± 2.44             |         |
| Group 2 (P1) | 86.0 ± 2.94             |         |
| Group 3 (P2) | 84.7 ± 2.87             | <0.001* |
| Group 4 (P3) | 78.7 ± 8.61             |         |
| Group 5 (P4) | 41.5 ± 10.4             |         |
| Group 6 (KP) | 10.0 ± 1.82             |         |

Parasol cream) far less than the P4 group (0.75% cream). This means that 0.75% black cumin extract cream is effectively used to reduce the amount of melanin pigment, but the effect produced is not as good as the Parasol cream.

DISCUSSION

Black cumin oil and seeds have been used extensively for centuries to treat various diseases in traditional Indian medicine such as Unani and Ayurveda (Goreja, 2003). Volatile oil, including thymoquinone, accumulates in the inner layer of the seed coat, so extraction cannot be directly from whole black cumin. Still, it is necessary first to destroy the skin (Botnick et al., 2012). Many active components have been isolated from black cumin and their oils including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigelicine, nigellidine and alphahedrin. Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) (Al-Ali et al., 2008). In a study, it was found that Thymoquinone, Carvacrol, t-anethole and 4-terpineon showed very significant scavenger (reduction) of radical compounds. These four elements and essential oils have variable antioxidant
activity when tested in the diphenyl picrylhydrazyl test for hydrogen atoms or unspecified electron donor activity. The compound is also known to be effective as a hydroxyl radical cleaning agent in testing non-enzymatic lipid peroxidation in liposomes and deoxyribose degradation tests (Sudhir et al., 2016).

Thymoquinone (TQ) has potential as a powerful antioxidant by regulating the reaction of reactive oxygen species (ROS) formation. TQ acts as a free radical scavenger. The TQ compound acts as a powerful antioxidant by inhibiting lipids and effective peroxidation of superoxide radical production (Gupta, 2016). Besides, TQ also increases the activity of several enzymes, such as catalase (CAT), superoxide dismutase (SOD), glutathione transferase, and glutathione (GSH). After reaction with antioxidant enzymes (GSH, NADH, and NADPH), TQ will produce two powerful antioxidants, namely glutathionyl-dihydrothymoquinone and di-hydro thymoquinone (Gupta, 2016).

One of the critical factors in evaluating the potential of sunscreen products is the value of the Sun Protecting Factor (SPF). Parasol Face Sunscreen Cream is a cream that has been proven to have a good SPF in protecting against sunlight. In one study, cream with 0.50% Nigella sativa oil was tested to determine the ability of the sun protection factor in vitro. It was observed that the formulation of Nigella sativa cream with a concentration of 0.50% had an SPF value of 1.05, which means 0.5% cream was considered to have a real sunscreen activity (Sudhir et al., 2016). This study found that the actual effect of black cumin extract cream as a new sunscreen was found at a higher concentration, namely at a concentration of 0.75%. In general, it can be concluded that black cumin extract cream with a concentration of 0.75% has a potential protective effect against UVB rays as a better sunscreen compared to a cream concentration of 0.25%, 0.5% or basic cream. However, this protective effect is still not as good as Parasol Face Sunscreen cream.

CONCLUSIONS

The administration of black cumin extract (Nigella sativa) with a topical concentration of 0.75% is effective as a sunblock to significantly reduce the amount of melanin pigment in male Wistar rats exposed to ultraviolet-B light.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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