EFFECT OF LICORICE (Glycyhrriza glabra Linn.),
A SKIN-WHITENING AGENT ON BLACK MOLLY
(Poecilia latipinnaa)

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Summary

Glycyhrriza glabra Linn. commonly known as Licorice, is a traditionally herbal remedy with an ancient history for its world wide usage in herbal preparations as a tonic, expectorant, demulcent, mild laxative and for allaying cough. It is also used as a depigmentation agent in cosmetics. Peoples in East Asia particularly females desire to keep skin white. To satisfy this desire many cosmetic companies have been developing melanogenesis inhibitors and discovering skin-whitening cosmetic preparations. Therefore, in this investigation Glycyhrriza glabra was evaluated for the inhibitory activity on tyrosinase and chronic fish toxicity test using Black molly fish model. The 50% Tyrosinase inhibitory concentration of Licorice extract was 34.48 µg/ml.
No Observed Effect Concentration (NOEC) for Black molly exposed to Licorice in portable water was obtained by 1mg/L, first observed effect concentration (FOEC) was obtained by 4 mg/l. In the present study the toxicity of Licorice extract was clearly observed in the liver sections and the bioaccumulation of the extract were increased with an increase in the concentration of Licorice extract.

Riassunto

Glycyhrriza glabra Linn. è un rimedio naturale tradizionale usato in tutto il mondo come tonico espettorante nelle forme influenzali, e svolge anche una leggera azione lassativa. Talvolta è utilizzata in cosmesi come depigmentante, soprattutto nel Sud-East Asiatico dove le donne desiderano mantenere la loro pelle particolarmente bianca.
A tal proposito, molte aziende cosmetiche hanno studiato e sviluppato preparazioni cosmetiche ad effetto depigmentante.
Per questo motivo è stata valutata l’attività della Glycyhrriza glabra Linn come inibitore della tiro-sinasì utilizzando come modello un particolare pesce nero.
La concentrazione che inibisce il 50% dell’attività tirosinasica è stata pari a 34.48 mg/ml. Con la concentrazione di 1 mg/L dell’estratto di liquirizia non sono stati ottenuti risultati positivi che, al contrario, si sono verificati con la concentrazione di 4 mg/L. Per quanto concerne la tossicità dell’estratto di liquirizia, si è notato che tende ad accumularsi nel fegato e che tale accumulo sembra essere direttamente correlato all’incremento della concentrazione utilizzata.
INTRODUCTION

Licorice (Glycyrrhiza glabra Linn.), a traditional herbal plant and its roots are widely used as flavoring agent in food and candy. It has been also employed in herbal preparations as a tonic, expectorant, demulcent, mild laxative and for allaying cough. Additional effects such as depigmentation, of Licorice was reported by Nodkarni (1991) and Tanyri et al. (1965). In India, it is reported to be cultivated in Baramulla, Srinagar Jammu, Dehra Dun, Delhi and South India (Sastri 1956) (Sharma et al. 2001).

Melanins are pigmented biopolymers that impart skin typology and tan. They are synthesized by the dendritic melanocytes dispersed at the dermo-epidermal junction. Melanin synthesis takes place in membrane bound organelles termed melanosomes, which contain specific enzymes controlling the production of the pigments. The first and rate-limiting step of melanin formation is mediated by tyrosinase (Hearing 1999; Ortonne & Ballotti 2000). Increased melanin synthesis or uneven distribution cause local pigmentation in the skin. Pigmentation disorders are caused by various factors including UV radiation, due to the destruction of the ozone layer. Excessive exposure to UV radiation may cause post-inflammatory pigmentation or hyper pigmentation (Kubo & Matshida 1995).

East Asia peoples, particularly the females desire to keep the skin white. To satisfy this desire many cosmetic companies have been developing melanogenesis inhibitors and discovering skin-whitening cosmetic preparations. In cosmetic preparations tyrosinase inhibitors such as Kojic acid, Arbutin, Ascorbic acid and Licorice extract have been exploited as whitening ingredients.

Today’s cosmetics consumer is a smart shopper. While attractive packaging is still an important factor in product appeal, the cosmetic user expects skin care formulations that are elegant, safe and live up to the consumer’s expectation of effectiveness. It is becoming increasingly apparent in the competitive field of cosmetics, that efficacy and safety claims are powerful product marketing tools. Unfortunately, statistically significant demonstrations of comparative clinical efficacy and safety of these products are not always met.

Safety assurance is one of the most important requirements routinely used by healthy people without medical supervision. In general an application for the approval of a new chemical as a cosmetic ingredient in globally must be accompanied with an extensive safety data such as acute toxicity, primary skin irritation, repeated skin irritation, sensitization, photo toxicity, photo sensitization, eye irritation, mutagenicity and human patch test, in order to obtain the approval of the Ministry of Healthy and Welfare (Itagari et al. 1995).

Only very limited level of studies for the environmental impact assessment is recommended for cosmetic ingredients, since environment issues are becoming recognized as a potential concern for consumer products that are eventually washed “down the drain”. Acute fish toxicity test is one, which is used to identify very high environmental effect concerns. Therefore, in the present investigation G. glabra was evaluated for its inhibitory activity on tyrosinase and chronic fish toxicity using Black molly fish model.

MATERIALS AND METHODS

Extraction

Licorice roots (Glycyrrhiza glabra Linn) were obtained from the commercial market. The roots were dried well at 37° C and powdered. Root powder (20 g) was soaked with 80 g of Propylene glycol (PG) solvent for 24 hr.
Followed by filtration, the filtrate was evaporated to dryness under vacuum. About 95% yield was collected and used for the evaluation of inhibitory activity of tyrosinase, Dopa autoxidation and toxicity study.

**Animals**

Total of 30 intact male black molly (Poecilia latipinna) weighing 3.01 to 3.03 g were chosen for the present study. Experimental fishes were obtained from Live Stock Research Institute, Kattupakkam, Chennai, Tamil Nadu, and were permitted to acclimate to laboratory conditions for at least one week prior to use. The animals were maintained in five groups of six (as duplicates) in slate bottomed glass aquaria (12 X12 X 18) inches) (Chavin 1963) under constant conditions of temperature (37° C), photoperiod (12 hrs per day) and diet.

**Chronic toxicity**

The test compounds were fed in 25 Liters of portable water (at a compound concentration of 25,50 & 100 mg of Licorice extract / 25 Liters). As a control group, the fishes were fed with Green tea powder (100 mg of Green tea powder / 25 Liters).

**Histochemical analysis**

Liver organs dissected from different groups were preserved in 16% buffered formalin. A 5-7µm thickness sections were stained with haematoxylin & eosin and photographed under 12.5 X magnification (McManus & Moowry 1956).

**Tyrosinase inhibition**

Tyrosinase activity is generally determined by spectrophotometry. The procedure followed that described by modified Vanni et al. (1990). In control tube 235 µl of 3 mM tyrosine and 285µl of 0.1 M phosphate buffer (pH 6.8) were added and incubated for 10 min at 25°C. Followed by incubation, 180 µl of mushroom tyrosinase (90 Units) from Sigma was added then incubated for 20 min at 25°C. For test samples, test solutions were added and buffer volume was adjusted accordingly. The pink color formed (Dopachrome) was measured as absorbance at 475 nm at the end of 20 min for each tube.

**Inhibition of Dopa autooxidation**

In case of control tube 250 µl of DOPA (4mM) and 200µl of Riboflavin (26 µM) in 550 µl of Phosphate buffer (0.05 M) was incubated under fluorescent lamp for 15 min. In case of test samples, buffer and test solution were adjusted accordingly. Color developed was measured at 475 nm, following the procedure of modified Joshi et al. (1987).

**RESULTS**

Figure 1 illustrated the toxicity of Licorice, and the mortality of test animals studied. It has been observed, Licorice at 4mg/l exhibited 100% mortality on day 7 of experimental period, whereas, the lower concentrations showed about 17% on day 25 for 1.0 mg /l concentration and 34% mortality on day 15 for 2.0 mg/l concentration. For Group - I (1mg/L), the remaining fishes were alive even after 45 days and for Group - II (2mg/L), the remaining 66% mortality was observed on 21st day. Group - III (4mg/L), fed uptake was observed to be reduced after three days. In the case of negative (Group - IV) and positive (Group - V) control experiments, no mortality and no reduction in fed uptake was observed throughout the experimental period. Figure 2 emphasizes the morphological changes observed in terms of body weight analysis carried out during the experimental period. The percentage reduction in body weight increases as the concentration of the test compound increases. At lower concentration, about 2.645% reduction was observed with group-I animals.
and it has been increased to 5.94% with group-II animals and 11.96% with group-III animals. Both the control groups were exhibited about 29 - 50% gain in body weight as illustrated in the Figure.

Fig. 1 Percentage mortality of Black Molly (Group - I (1mg/L); Group - II (2mg/L); Group - III (4mg/L); (Group - IV and V) negative and positive control).

Fig. 2 Change in body weight with respect to concentration of Lecorice on Black Molly (Group - I (1mg/L); Group - II (2mg/L); Group - III (4mg/L); (Group - IV and V) negative and positive control).
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**Histochemical analysis**

Examination of haematoxylin and eosin stained sections of liver tissues, for the experimental animals at the time of exposure to the test compound exhibited that the degree of damage increases with an increase in the concentration of the test compound. Similar to the mortality and the reduction in body weight, higher the concentration applied higher the tissue damage was observed. Figure 3a & 3b illustrates the H&E staining of liver tissue obtained from control groups. (Group –IV & V). It has been observed that there was a high level of viable cell architecture pattern with regenerative changes with mild hepatocellular swelling, cholestasis and moderate micro and macrovesicular steatosis with no inclusion of foreign body. Moreover, chronic mural inflammation with non regenerative pattern suggests that the injury to the hepatic cell was only due to the test compound supplied. In addition, inclusion of foreign body was evidently exhibited in Figures 4a, 4b and 4c.

**Tyrosinase inhibition assay**

*In vitro* analysis carried out to assess the 50% enzyme inhibitory concentration of the test compound. Figure 5 illustrated that, about 50% inhibition of tyrosinase activity was exhibited by the Licorice extract was 34.48 µg/ml, compared to the positive test control carried out with kojic acid at 2.0 µg/ml.
DISCUSSION

Cosmetic industries are one of the fast-growing industries. Advent of new cosmetics with new formulations is a great challenge to the cosmetic industries. Most of the new cosmetic products are the different combinations of old ingredients. Salminen (2002) states that, there has been a significant trend in using new ingredients to give cosmetics unique properties not available from the standard battery of old ingredients. Interest in discovery of new skin-lightening agents is currently on demand by the cosmetic, consumer product and pharmaceutical industries (Petit & Pierard 2003). This situation corresponds to a perceived need in the market place for novel agents with increased efficacy and improved safety profiles. Thus, new compounds are frequently appearing in cosmetic industry trade journals. But, the product owners have not statistically proved the clinical efficacy and the safety in usage of the products. Lack of clinical trails betray the products usage.

Chronic effects have been the focus of most recent toxicity studies. The No Observed Effect Concentration (NOEC) for Black molly (Test animal in the present study) exposed to Licorice in portable water was 1 mg/L, first observed effect concentration (FOEC) was 4 mg/L. In the present study the toxicity of Licorice extract was clearly observed in the liver sections and the bioaccumulation of the extract were increased with the increase in the concentration of Licorice extract.

Various functions of crude licorice extracts have been shown over many years (Takagi & Ishii 1967). Glycyrrhizin and glychrrhetinic acid are the main constituents of the hydrophilic fraction of licorice extracts and are known to be anti-inflammatory agents (Inoue et al. 1986). The hydrophobic fraction of licorice extracts, which contains various flavanoids, has been know to have the inhibitory effects on melanogenesis due to its inhibition of tyrosinase activity (Kameyamma et al. 1994) and in the similar way Glabridin (oil soluble fraction of licorice) also has been known to have the inhibitory effects on melanogenesis. In addition, contact allergic dermatitis can also be developed by the
application of these compounds (Nishioka et al. 1999). Moreover, consumption of licorice of 10-45 g/day causes raised blood pressure together with a block of aldosterone, rennin and electrocardiogram changes (Newall et al. 1996). The 50% Tyrosinase inhibitory concentration of Licorice extract was 34.48 μg/ml, earlier similar type of result was reported by Khanom et al. (2000). The chosen extract does not have any action on reducing or inhibiting the Dopa autooxidation reaction even at 500 mg/ml, earlier similar type of result was reported by Lee et al. (1997).

**CONCLUSION**

Skin care products based on natural materials needs high clinical efficacy and safety. Effect of Licorice extract on inhibition of tyrosinase activity on Black molly showed in vitro 50% inhibition on comparison with standard inhibitor kojic acid, but the accumulation of extract in the liver tissue showed damaging effects and toxicity.
References


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