Studies on Isolation, Purification and Characterization of Alpha-linolenic Acid of Tasar Pupae Oil

A Summary of the Thesis Submitted to the
Babasaheb Bhimrao Ambedkar University, Lucknow
in Fulfillment of Requirement for the Award of Degree of

Doctor of Philosophy
in
Zoology

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(A CENTRAL UNIVERSITY)
(ACCREDITED 'A++' GRADE BY NAAC-2023)
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2024

In India, the sericulture industry plays a remarkable role in the Indian economy as it produces five different types of silk namely, mulberry, eri, muga, tropical tasar, and temperate tasar. Nowadays, the sericulture industry is also playing a significant role in the biomedical area, as the by-products of this industry, which were earlier considered as seri-waste, have now revealed excellent therapeutic value. Investigations on various by-products of sericulture industry such as larva, excreta, pupae, pupae oil, chitin, etc. have shown that these co-products have diversified biological activities, including hepatoprotective, anti-oxidant, anti-diabetic, neuroprotective, anti-inflammatory, anticancer, antimicrobial, etc.

Previously, the pupae of silkworms were considered unproductive by-products of the sericulture industry. Nonetheless, researchers across the globe realized the importance of silkworm pupae when it was discovered that these pupae are tremendous source of bioactive constituents such as amino acids, peptides, lipids, and omega-3 fatty acids. Numerous fatty acids, including stearic acid, palmitic acid, oleic acid, linoleic acid, linolenic acid, and alpha-linolenic acid (ALA), are present in silkworm pupae, particularly ALA, which makes up a significant portion of them. ALA is an essential fatty acid widely known for its pharmacological properties, such as such as anti-diabetic, anti-inflammatory, anti-oxidant, neuroprotective, anticancer, anti-hyperlipidemic, cardioprotective, etc. According to studies, silkworm pupae protect the liver, boost the immune system, prevent tumour growth, fight bacteria, and regulate blood sugar and lipid levels, and lower blood pressure.

Silkworm pupae contain significant amount of oil content which is also rich in ALA, linolenic acid, oleic acid etc. Various studies have supported that silkworm pupae oil is suitable for consumption with added health benefits due to high amount of omega-3 fats. According to studies silkworm pupae oil is also an excellent therapeutic agent as it has exhibits various

pharmacological activities including antibacterial, anticancer, anti-oxidant, anti-hyperlipidemic, hepatoprotective etc. In addition, silkworm pupae oil was reported to be safe and effective on animal models according to investigations. Hence silkworm pupae oil is a novel source of lipid with amazing nutraceutical properties.

Pupae of various silkworm species such as Mulberry and non mulberry (eri and muga) are used to extract oil. However, pupae of tasar silkworm (*Antherea mylitta*) have been less studied for oil extraction and further analyses. Consequently, the aim of the current study was to extract, characterize, and analyse the edible and therapeutic properties of tasar silkworm pupae oil (TPO). Initially, the fresh tasar pupae were dried at 70ºC in hot air oven. Once the pupae were completely dried, they were converted into fine powder and powder was subjected to oil extraction using Soxhlet extraction method. Further, the extracted TPO was studied for physico-chemical characterizations, including peroxide, saponification, iodine, acid, pH, color, rheological, and viscosity values. The fatty acid profile of TPO was determined using Gas-chromatography Mass spectroscopy (GC-MS). Preparative High performance Liquid Chromatography (Prep-HPLC) was employed to extract ALA from TPO. The quantification of extracted ALA was done using Gas Chromatography Flame Ionization detection (GC-FID). Afterwards, the using the Sulforhodamine B assay anticancer property of TPO and extracted ALA was tested on cancer cells. In addition, the effect of TPO was studied on oxidative stress and serum cholesterol levels in streptozotocin-induced diabetic rats. Results of physicochemical characterization suggest the quality of TPO is similar to that of the other edible oils that are currently available in the market such as olive oil, mustard oil etc. This indicates that TPO is fit for edible purposes. GC-MS data showed that ALA was the dominant fatty acid (38%) in TPO. After the confirmation of ALA in TPO, successful extraction of ALA was established using

Prep-HPLC. The quantitative analysis of extracted ALA using GC-FID revealed that the ALA concentration was 11760 ppm (11.76 mg/ml). Afterwards, results of *in vitro* anticancer study showed that TPO and ALA drastically hampered the growth of human colon cancer cells (COLO-205) with a GI_{50} (Growth Inhibition) value of ≤ 20 µg/ml. Previous studies have shown that ALA and oils rich in ALA exhibit significant anticancer activities. Similarly, in the current study, we can infer that ALA might be responsible for the anticancer activity of TPO. Furthermore, results of an *in vivo* study revealed that TPO increased the levels of catalase (CAT) and superoxide dismutase (SOD) in diabetic rats. These anti-oxidant enzymes are known to scavenge free radicals in the body. Until now, no studies associated with the anti-oxidant properties of silkworm pupae oil on animal models have been recorded. To our knowledge, this was the first study on the effect of silkworm pupae oil on oxidative stress in diabetic rats. Additionally, TPO reduced the total serum cholesterol level in the diabetic rats. Previous researches also confirmed that silkworm pupae oil controls plasma lipid and lipoprotein levels in rats' serum.

In conclusion, the current study showed that TPO exhibits significant therapeutic as well as edible properties. TPO can be used in the future to mitigate cancer and diabetic-related complications that are being major concerns in human society. Moreover, TPO could be a novel source of healthy fat that can be used in the food and feed industry in place of unhealthy Trans fats and saturated fatty acids. In future additional research needs to done to fully investigate the further therapeutic activity of TPO for the betterment of the human health.