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Emerging Role of Silkworm Pupae in Pharmaceutical Industries

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Silkworm pupae are one of the major by-products generated in silk industry during reeling of silk. The industry generates a massive quantity of 2 kg dry pupae and 8.014 kg wet pupae from one kg of raw silk production and left over silkworm pupae posing significant disposal challenges. It is usually discarded into the open area thus, almost all the proteins and nutrients present in the silkworm pupae naturally spoiled by bacteria or fungi, hence, the nutritional quality of the final product is not in uniform, and these spoiled pupae are generally applied as fertilizer for crops and to some extent as a feed for fish in countries like Japan, Korea, and India. It is also used as food insects, food and feed in traditional medicine in Asian countries such as India (northeast), China, Japan, South Korea, and Thailand promoting circular production and environmental sustainability by lowering waste release.

Nutrient Composition of silkworm pupae

The proximate composition of silkworm pupae is presented in Table 1. Silkworm pupae contain 55.61 % of total protein and 18.20 % lipid content by dry weight. Silkworm pupae per 100 g sample contain various biochemicals such as 55 g protein, 8.5 g fat, 6 g fiber, 25.43 g carbohydrates, and 389.60 (Kcal/100 g) energy contents. Silkworm pupae also contain diverse mineral compositions (mg/100 g) such as 102.31 mg calcium, 1826.59 mg potassium, 287.96 mg magnesium, 1369.94 mg phosphorus, 274.57 mg sodium, 9.54 mg iron, 17.75 mg zinc, 2.08 mg manganese and copper, and 0.08 mg selenium.

Table 1. Proxmate composition of silkworm pupae

S.No.	Parameter	Silkworm pupae (%)
1	Moisture	75.00
2	Lipid	18.20
3	Crude protein	55.61
4	Crude fibre	0.82
5	Ash/minerals	7.30
6	Carbohydrates	16.20

(Source: Bandlamori *et al.*, 2019)

The biological active peptides, made up of amino acids, which are involved in several physiological functions. The proteins present in silkworm pupae are hydrolyzed and converted into several biologically active compounds responsible for pharmacological functions of the silkworm pupae. Silkworm pupae consist of 18 amino acid makeup and eight essential amino acids fulfill the WHO/FAO/UNU recommendations. There are another ten non-essential amino acids that are required by humans. Phenylalanine and proline levels in silkworm pupae are greater than in hen eggs. Amino acids like asparagine, threonine, glutamine, glycine, alanine, methionine, isoleucine, leucine, lysine, proline are more abundant in silkworm eggs as compared to hen eggs.

Silkworm pupae oil contains a large number of unsaturated amino acids, especially Omega-3 fatty acids. Silkworm pupae are also rich in polyphenols and vitamins include Vitamin A, B1, B2, B3, B5, B7, B9, B12, C, and E. Phospholipids and five tocopherols, which include α-tocopherol, β-tocopherol, γ-tocopherol, γ-tocotrienol, and σ-tocopherol are also present in silkworm pupae. Sadat et al. (2022) studied and enumerated the vitamin composition of mulberry silkworm pupae i.e., vitamin A (273.99 mg/100 g), vitamin B1 (1.91 mg/100 g), vitamin B2 (5.43 mg/100 g), vitamin B3 (15.20 mg/100 g), vitamin B5 (12.49 mg/100 g), vitamin B7 (144.51 mg/100 g), vitamin B9 (0.41 mg/100 g), vitamin B12 (500 mg/100 g), vitamin C (5.70 mg/100 g) and vitamin E (34.47 mg/100 g). The sugars found in silkworm pupae are biologically active, including chitosan, chitin, and isolated polysaccharides.

Numerous studies indicated that the bioactive compounds present in silkworm pupae have various pharmacological functions, such as antioxidant, antibacterial, anticancer, antiapoptotic, hypotensive, immunomodulatory, lipid- and blood sugar-regulating and hepatoprotective activities which provides a broader prospect for the pharmaceutical application of silkworm pupae are discussed below:

Antioxidant activity

Polyphenols and peptides present in silkworm pupae are responsible for antioxidant properties. Two peptides isolated from silkworm pupae have significant antioxidant function in HepG2 cells as evidenced by superoxide dismutase expression, ROS reduction and glutathione (GSH) production. The antioxidant activity in pupae differ by sex and age, with female pupae in the early stages of pupation showing greater free radical and ROS scavenging. It may be possible to produce foods or pharmaceuticals that have antioxidant properties because of the peptides, phenols, and unsaturated fatty acids in silkworm pupae (Cermeño et al., 2022).

Anti-bacterial activity

The growth of a *Staphylococcus sciuri* was strongly suppressed by silkworm pupa oil. Chitin and chitosan, which are abundant in silkworm pupae shells and have high antibacterial characteristics, are employed in a variety of biomedical applications. Chitosan in silkworm pupae was found to be 67% acetylated and 48% crystalline. The antibacterial and antifungal efficacy of silkworm pupae-derived chitosan was superior to that of commercially available chitosan, with the quickest bacterial suppression occurring at 1–2 h. Silkworm pupae contain an antibacterial substance that could be utilized to treat illnesses and cut down on the excessive use of antibiotics (Dev *et al.*, 2017).

Anticancer activity

In vitro studies have shown that the amino acids and protein hydrolysates in pupae have anticancer capabilities and are lethal to the cancer cells of the human breast, stomach, and liver. Silkworm pupae proteins influence the cancer cells' cycle of cell division and trigger the synthesis of apoptotic factors, which aid in apoptosis, acting as anticancer agents. Cancer cell mitochondria are affected by silkworm pupae protein, which in turn impairs the operation of their energy metabolism and triggers the apoptotic flux, which kills the cancer cells (Chukiatsiri *et al.*, 2020).

Antiapoptotic activity

Silkworm pupae are rich in a low-molecular-weight lipoprotein, a member of the 30 K family of proteins which transport lipids and inhibited apoptosis in mammalian cells. Haemolymph from silkworm pupae was found to suppress apoptosis in virus-infected insect cells and increase the longevity of the cells. Subsequently, a non-glycosylated monomeric protein with antiapoptotic activity was purified from the haemolymph (Koo *et al.*, 2009).

Hepatoprotective activity

Silkworm pupae oil increased the pH of the stomach in mice and reduced the size of peptic ulcers and gastric discharge. silkworm pupa's oil decreased serum levels of IL-6, IL-12, TNF-, IFN-, MTL, and GT in rats with gastrointestinal ulcers, while increasing levels of SST, SOD, GSH-Px, VIP, and CAT. While eNOS, EGF, EGFR, and VEGF expression were up-regulated, COX-2, iNOS, NF-B, and Bcl-2 expression were down-regulated. Silkworm pupae oil also reduced acetaminophen-induced acute liver injury and alcohol-induced hepatotoxicity and oxidative stress in mice by inhibiting the oxidative-stress-mediated NF-κB signalling pathway (Cha *et al.*, 2012).

Cardiovascular protection

30 K protein in silkworm pupae not only possesses the antiapoptotic activity, but also protective effects against cardiovascular disease. Silkworm pupae 30Kc6 protein reduced serum levels of total triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDLC), and total cholesterol (TC), and reduced the extent of aortic and liver lesions in atherosclerotic rabbits. Crude extracts of silkworm pupae have also been reported to improve the condition of atherosclerotic rabbits, presumably through antioxidant and hypolipidemic effects. Silkworm pupae oil sodium salt was found to reduce platelet-derived growth-factor-induced abnormal migration and proliferation of vascular smooth muscle cells. Silkworm pupae oil sodium salt treatment down-regulates ERK1/2 phosphorylation levels in vascular smooth muscle cells (Kim *et al.*, 2020).

Other biomedical applications

- Pupal oil can effectively reduce triglycerides, prevent and treat fatty livers protect the liver after consumption of alcohols, improve the blood quality and the environment within the blood vessel, effectively soften the blood vessels, lower blood pressure, and prevent arteriosclerosis and thrombosis.
- Pupal oil enables the prostaglandins to maintain balance with effects of preventing prostate diseases, improving the functions of insulin-producing beta cells, restoring the fatty acid desaturase activity of cells in diabetic patients and has marked hypoglycemic effect free from reoccurrence
- The natural steroids present in the oil can improve fertility and enhance sexual function; the unsaturated fatty acids in the oil which cannot be synthesized by humans can enhance the flexibility of immune cell membrane, increase the vitality of the immune cells, so that the barriers to human health are more robust, and the occurrence of sub-health and disease is effectively prevented.
- The α-linolenic acid and other active substances contained in pupal oil can join the synthesis of human tissue cell membranes, effectively prevent and mitigate symptoms like wrinkles, pigmentation, sallow skins, and premature aging of modern women. It can improve the body superoxide dismutase activity and decrease free radical with good antiaging effects.

Conclusion

Silkworm pupae, major by product obtained after reeling the silkworm cocoons which are disposed as a waste into environment and cause detrimental effects. Silkworm pupae are rich in proteins, carbohydrates, vitamins, minerals, phenols, chitin, chitosan and other polysachharides whicha re responsible for biological properties like antioxidant, antibacterial, anticancer, antiapoptotic, hypotensive, immunomodulatory, lipid- and blood sugar-regulating and hepatoprotective activities provides a broader future prospect for the pharmaceutical applications which helps to generate additional income to the reelers and make the sericulture industry economically viable.

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