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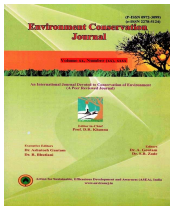

In vivo studies on anti hyperglycemic activity of sericin using rat model

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ABSTRACT

Silk protein, sericin was isolated from raw silk fabrication in reeling industry and subsequent process of silk throwing. The majority of it is thrown in the waste water from silk manufacturing. Sericin was used in antibacterial, antioxidant, wound healing, moisturising, and antiaging medications and cosmetics. Limited reports on the use of sericin in the treatment of diabetes prompted the current study on the use of sericin in the control of diabetes in rats. Thus, the present study was aimed to investigate the antihyperglycemic activity of sericin protein using rat model. The results indicated that sericin treated groups showed a noteworthy augment in body weight than the standard drug (Metformin) treatment with diabetic control. Sericin @ 400mg/kg showed 152.6 mg/dl and 141.2 mg/dl on 7th and 21st day of treatment. Similarly, Sericin @ 800mg/kg showed 126.0 mg/dl and 114.0 mg/dl on 7th and 21st day of treatment when compared to control (206.0 mg/dl and 203.6 mg/dl). SGOT of 68.4 and 50.0 units per litre of serum was observed in sericin @ 400 mg/kg and 800mg/kg treated rats when compared to control 138.6 units. SGPT 0f 26.0 and 21.6 units per litre of serum was observed in sericin @ 400 mg/kg and 800mg/kg treated rats when compared to control (75.0 units). Triglycerides of 96.4 and 81. 4 units were observed in sericin @ 400 mg/kg and 800mg/kg treated rats when compared to control (152.8 units).

Introduction

Sericin, protein produced by silkworm, *Bombyx mori* during the construction of silk (Padamwar and Pawar, 2004). Two proteins: fibroin (70–80%) and sericin (20–30%) obtained from silk is spun by mature fifth instar larvae of the silkworm *Bombyx mori* (L.). The primary core protein is fibroin, and the gum-like covering that surrounds it is sericin. Sericin is made up of 18 different amino acids, with 32 percent of the serine found as a randomised amorphous coil. Sericin can be easily transformed into a -sheet conformation in the amorphous coil by repeated moisture absorption and mechanical stretching (Mondal *et al*., 2007) (Mondal *et al*., 2007). The layers on top of the fibrin are made up of three different forms of sericin (Takusu *et al.,* 2002; Lalit Jaipura, 2015). The topmost layer, Sericin A, is insoluble in water and contains around 17 percent nitrogen, as well as amino acids like serine, threonine, aspartic acid, and glycine. Sericin B, which makes up the middle layer, is similar to

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sericin A but additionally contains tryptophan. The innermost layer, sericin C, is the layer that is nearest to and contiguous to fibroin. Sericin C, which is also insoluble in water, can be extracted from fibroin by using a hot, weak acid. Sericin C contains all of the amino acids found in Sericin B, plus proline (Aramwit *et al.,* 2012).

Around one million tonnes of fresh weight cocoons and 4,00,000 tonnes of dried cocoons are produced worldwide, yielding around 50,000 tonnes of recoverable sericin. Every year, India produces roughly 1600 tonnes of silk, leaving behind approximately 250-300 tonnes of sericin (Ghosh *et al*., 2019). The majority of silk sericin is eliminated during the reeling industry's raw silk manufacture and subsequent phases of silk tossing. Silk sericin is currently primarily thrown in waste water from silk production. Silk sericin can provide a major economic, scientific, and societal advantage if it is recovered and recycled.

Antimicrobial, antioxidant, wound healing, bioadhesive moisturising, antiwrinkle, and antiaging are only a few of the uses for sericin in pharmaceuticals and cosmetics. Because of its antibacterial and UV resistant qualities (Gupta and Agarwal, 2014), sericin is employed as a biomaterial (Chirila *et al*., 2016). Sericin from the cocoons of the mulberry silkworm, *Bombyx mori* (L.), and the non-mulberry, tropical tasar silkworm, *Antheraea mylitta* (L.), has been shown to have antioxidant properties in skin fibroblast cell lines exposed to hydrogen peroxide for 24 hours (Vittalrao, 2016; Khayade *et al*., 2016). Song *et al.* (2015) investigated the effects of sericin on the testicular growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis in rats with type 2 *Diabetes mellitus.*

Most diabetic patients manage their blood glucose levels using allopathic medications, but liver and kidney damage develops quickly, and some diabetics lose their ability to participate in routine daily activities in a short period of time (Shiyovich *et al*., 2010; Russell, 2010). As a result, diabetic patients require medications with low toxicity and side effects. Sericin is a prospective drug candidate that possesses the qualities listed above. Rare reports on the use of sericin in the treatment of diabetes inspired the current study on the use of sericin for diabetes control in rats.

Material and Methods

Procurement of animals

PSG Institutional Animal House Facility, PSG College of Pharmacy, Coimbatore provided Albino male Wistar rats aged 5 to 7 weeks with a body weight of 180-200 g for the study. The rats were fed a regular meal and given unlimited access to water for 7 days before being acclimatised to laboratory settings. All rats were housed in separate cages and fed and watered daily for up to 6 weeks before being used in the study. All of the groups were housed in a temperature-controlled environment with humidity of 55% and a temperature of 24°C 1°C. The rats' body weights and blood glucose levels were measured on a weekly basis and percent change in body weight was calculated. The experiment was carried out with the permission of the Institutional Animal Care and thic Committee at PSG College of Pharmacy in Coimbatore (Approval licence No. 442/IAEC/2019).

Induction of diabetes in rats

After acclimatization the animals were subjected to the induction of diabetes. For diabetes induction 250 mg of STZ was prepared in 0.1 M freshly prepared citrate buffer pH 4.5. The animals were kept under over night fasting. The following morning, they were injected with STZ at a dose of 30mg/kg *via* intraperitoneally route in single dose. For the first 24 hours, animals were provided with 10% sucrose solution to prevent shock due to hypoglycemic episodes. Blood glucose levels were monitored at 0, 3 and 5 hours after STZ injection by using Glucosphera blood glucose monitoring system. Subsequently, the blood glucose levels were monitored at 2 days interval after the STZ injection. On day 14, animals with fasting blood glucose level > 180 mg /dl were selected for the study and randomized to control and treatment groups.

Experimental Design

The animals were randomly divided into the four experimental groups with 6 animals per group. Group 1 received vehicle; group 2 received the standard drug Metformin (75mg/kg/day); group 3 received sericin@ 400 mg /kg/day and group 4 received sericin @ 800 mg/ kg/ day for 21 days through oral route.

Effect of sericin on body weight and fasting blood glucose and biochemical parameters

The body weight of rats of each group was measured at the day of 1, 7 and 14.The fasting blood glucose was measured at days 1,7,14 and 21. At day 21, the animals were sacrificed with a high dose of ketamine and blood was collected by carotid cutting. The plasma was isolated and stored at -20^0 C for further biochemical analysis. The fasting blood glucose level of rats in each group was measured by using Avantor blood glucose monitoring system.

Preparation of samples for biochemical assay

Plasma was extracted from blood samples centrifuged at 3000 rpm for 20 minutes. The samples were stored at –20 °C until they were tested, and any red blood cells that remained on the bottom of the tubes were washed with a phosphate buffer, pH 7.4, and the samples were kept at –20 °C until they were analysed. Coagulation was allowed on freshly obtained blood. Blood was centrifuged at 3000 rpm for 15 minutes after it coagulated, roughly 2 hours after it was collected, to extract blood serum. Serum was transferred to an Eppendorf tube and centrifuged for 10 minutes at 1000 rpm. Serum samples were transferred to new Eppendorf tubes and stored at -20°C until further analysis. Parameters *viz.,* SGOT, triglycerides, cholesterol level of rats in each group was measured by using Agappe biochemical kit.

Statistical analysis

The mean and standard error of the mean were used to express all of the data (S.E.M.). Statistics was carried out using TWO WAY ANOVA followed by Bonferroni post test.

Results and Discussion

In vitro and *in vivo* investigations of sericin's biocompatibility and antioxidant capability proved that it is immunologically inert, as well as proving its safety and opening up a wide range of biomedical applications (Sehnal, 2008 ; Lamboni *et al*., 2015). Sericin has also been shown to treat diabetes (Yang *et al*., 2002) decrease cholesterol (Limpeanchob *et al.,* 2010) and activate the immune system (Panilaitis, 2003).

Effect of sericin on body weight

Percentage change in body weight was measured for each group on day 1, 7 and 14th day when compared with day 1 for all the groups. Data were expressed as mean ± SD (n=5). There was a significant increase in the body weight of sericin treated groups at the P< 0.05 compared with standard treatment group and P<0.01 on comparison with disease control group. Sericin treated groups showed a significant increase in body weight than the standard drug (Metformin) and disease control. These findings indicated that sericin has an anabolic activity in diabetes associated weight loss.There was a significant increase in the body weight of sericin treated groups when compared with standard treatment group and when comparison with diabetic control group (Table 1).Treatment of streptozotocin-induced diabetics with sericin at a dose of 800 mg/kg body weight resulted in a substantial increase in glucose level of 114 units, followed by treatment with sericin at a dose of 400 mg/kg body weight resulted in a glucose level of 108.24 (11.786) units. In streptozotocin-induced diabetic mice, Rattana *et al*. (2017) studied the antihyperglycemic activity of silkworm powder, fibroin, and sericin isolated from three races of Thai silkworm (Nangnoi, Nanglai, and Samrong). The results showed that diabetic rats given sericin gained a significant amount of weight throughout the course of the experiment, approaching that of normal control rats, implying that sericin is effective in reducing the severity of the condition. Body weight loss may result via glycolysis, lipid, and protein metabolism in muscle to obtain sufficient energy, which is caused by insulin-mediated glucose uptake resistance in peripheral tissues, preventing glucose from being used as a major energy source (Ravi *et al*., 2004).

Effect of sericin on Fasting Blood Glucose (FBG) level

The fasting blood glucose (FBG) of each group of animals was assessed on days 1,7,14 and 21. The FBG of each group of animals was assessed. Groups treated with sericin showed a significant increase in FBG compared to standard drug (metformin) treatment and diabetic control. Change in FBG was measured for each group on day 1,7,14 and 21 and compared with day 1 for all groups. Sericin showed a significant increase in FBG compared to standard drug treatment (Fig.1).

Table 1. Effect of Sericin on body weight and FBG in diabetic induced rats

Groups	Body weight (%)			Fasting Blood Glucose (FBG)		
	1st day	7th day	14th day	1st day	7th day	14th day
Group 1	100	93.01	85.56	207.40	206.00	204.60
Group 2	100	92.54	86.59	202.00	116.40	115.60
Group 3	100	97.62	99.55	200.60	152.60	148.20
Group 4	100	96.56	103.40	200.80	126.00	111.40

Table 2. Effect of sericin on biochemical parameters of diabetic induced rats

Biochemical attributes	SGOT (units/litre of blood serum)	SGPT (units/litre of blood serum)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Group 1	138.60	75.00	170.60	152.80
Group 2	76.80	40.40	137.20	117.00
Group 3	68.40	26.00	107.00	96.40
Group 4	50.00	21.60	105.60	81.40

Sericin @ 400mg/kg showed 152.6 mg/dl and 148.2 mg/dl on 7th and 14th day of treatment. Similarly, Sericin @ 800mg/kg showed 126.0 mg/dl and 111.40 mg/dl on 7th and 14th day of treatment when compared to control (206.0 mg/dl and 204.6 mg/dl). Fasting glucose level in three Thai silkworm races were studied by Rattana *et al*. (2017. The results revealed that fibroin and sericin extracted from Nangnoi race expressed a better reduction of FPG in diabetic rats compared with diabetic control groups. Mice fed silk protein (fibroin and sericin) had lower insulin levels and higher glycogen concentrations than mice fed high fat. In mice, studies on silk protein revealed that the soluble fibroin could lower blood glucose and boost insulin levels. Okazaki *et al*. (2010) discovered that dietary administration of silk sericin reduced plasma glucose and enhanced insulin production in high fat-fed rats after an interperitoneal glucose injection. In the Brown Rat, Rattus norvegicus, Khyade and Pahade (2018) employed an aqueous solution of sericin to treat diabetes. The results showed that treating streptozotocin-induced diabetics with an aqueous solution of sericin at a dose of 250 mg / kg body weight resulted in a rise in glucose level of 142.53 (26.081) units, while a dose of 500 mg / kg body weight resulted in a glucose level of 108.24 (11.7786) units. The glucose level was lowered as the dose of sericin aqueous solution was increased. Sericin and flavonoids are two bioactive compounds that have been shown to have a therapeutic impact on diabetic nephropathy. The effect of ethanolic extract (EE) from the green cocoon of the silkworm Bombyx mori on DN in type 2 diabetes (T2D) mice generated by high-fat and streptozotocin (STZ) was examined by Wang et al. (2019). When compared to a negative control, the results showed that EE administration reduced blood glucose levels and improved body weight in diabetic mice. Oral EE could inhibit the expressions of renal tumour necrosis factor TNF-, monocyte chemoattractant protein-1 (MCP-1), fibronectin (FN), and P38 mitogen-activated protein kinase (p38 MAPK) in T2D mice. Furthermore, T2D mice treated with EE had considerably higher levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-px).

Effect of sericin on biochemical parameters

Group treated with sericin 800mg/kg showed a significant decrease in SGOT compared to standard drug treatment and disease control. SGOT of 68.4 and 50.0 units per litre of serum was observed in sericin @ 400 mg/kg and 800mg/kg treated rats when compared to control 138.6 units). Changes in SGOT level was measured for each group on day 21 and compared with day 1 for all groups (Fig 3). Changes in the SGPT levels were measured for each group on day 21 and compared with day 1 for all groups. SGPT 0f 26.0 and 21.6 units per litre of serum was observed in sericin @ 400 mg/kg and 800mg/kg treated rats when compared to control (75.0 units) (Fig 4).The sericin treated group 800mg/kg showed a significant decrease in triglycerides followed by sericin 400mg/kg treated group showed a significant decrease in triglycerides when compared to standard drug therapy and disease control groups. Triglycerides of 96.4 and 81. 4 units were observed in sericin @ 400 mg/kg and 800mg/kg treated rats when compared to control (152.8 units) (Table 2) (Fig 5). The cholesterol levels of each group of animals were assessed (Fig 6). The sericin treated group 800mg/kg showed a significant decrease in cholesterol and sericin 400mg/kg treated group showed a significant decrease in cholesterol compared to standard drug and disease control.

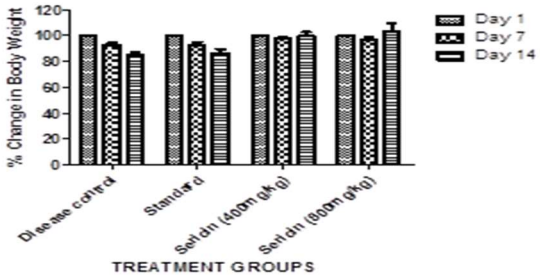

Figure 1: Effect of sericin on body weight in diabetic induced rats

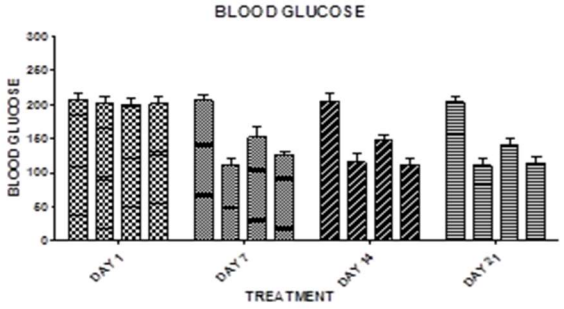

Figure 2: Effect of sericin on blood glucose level in diabetic induced rats.

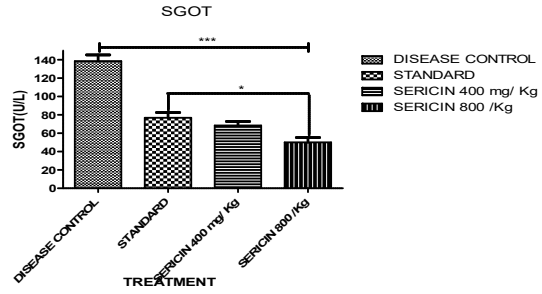

Figure 3: Effect of sericin on SGOT in diabetic induced rats

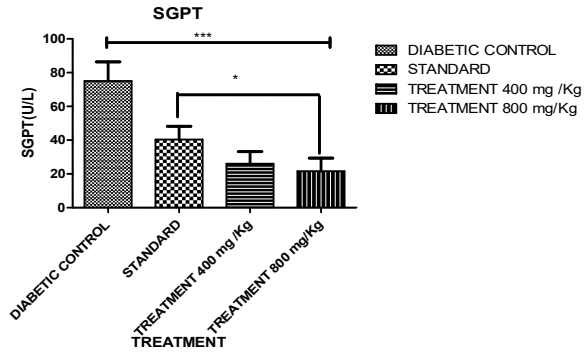

Figure 4: Effect of sericin on SGPT in diabetic induced rats

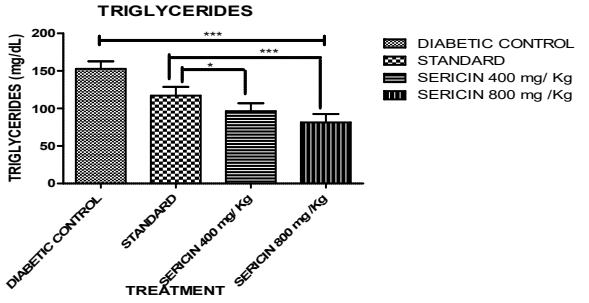

Figure 5: Effect of sericin on triglycerides in diabetic induced rats.

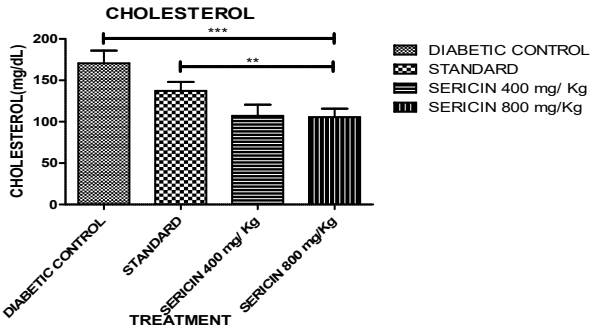

Figure 6: Effect of sericin on cholesterol in diabetic induced rats.

Changes in cholesterol levels were measured for each group on day 21 and compared with day 1 for all groups. Sericin @ 400 mg/kg and 800mg/kg treated rats showed cholesterol level of 107. 0 and 105.6 units when compared to control showed 170.6 units. Limpeanchob *et al*. (2010) studied the cholesterol-lowering impact of sericin in rats given cholesterol with and without sericin for 14 days. In rats fed a high-cholesterol diet, all three dosages of sericin lowered non-high-density lipoprotein (HDL) and total serum cholesterols (10, 100, and 1000 mg kg-1 day-1). The absorption of radiolabeled cholesterol into differentiated Caco-2 cells and cholesterol solubility in mixed lipid micelles was used to investigate the putative mechanism of action. Sericin concentrations as low as 25 and 50 g/mL prevented 30% of cholesterol uptake in Caco-2 cells, although larger concentrations had no impact. In the presence of sericin, cholesterol micellar solubility was reduced. The cholesterol-lowering action of sericin is thought to be due to its suppression of cholesterol absorption in intestinal cells and reduction of cholesterol solubility in lipid micelles, according to this study. Dietary sericin

lowered serum levels of triglycerides (33%), cholesterol (16%), phospholipids (18%), and free fatty acids (18%) considerably (27 percent). The sericin intake also lowered serum VLDL-triglycerides, VLDL-cholesterol, LDL-cholesterol, and LDL-phospholipids. The decrease in serum triglycerides appeared to be due in significant part to the decrease in VLDL triglycerides (Okazaki *et al*., 2010). The decrease in blood triglycerides results in a decrease in very low-density lipoprotein (VLDL) levels without impacting serum high-density lipoprotein levels (Kato, *et al* , 2002). Because a high triglyceride and VLDL level raises the risk of atherosclerosis, sericin use can help prevent atherosclerosis. Sericin also inhibits the buildup of lipids in the liver and reduces the release of triglycerides into the bloodstream.

Conclusion

Intake of allopathic diabetic medications causes liver and renal impairments in the early stages, as well as a loss of ability to participate in normal daily activities in the later stages. Silk proteins have a variety of biological and pharmacological roles, including antioxidation, antidiabetic, anti-cancer, anti-inflammatory, and antibacterial properties. The current investigation demonstrated that sericin might be employed as a possible diabetes medication option with no toxicity or side effects. However, more research into the bioactive characteristics of sericin for the development of diabetic drugs is required.

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Conflict of interest

The authors declare that they have no conflict of interest.

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