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Utilization of Aqueous Solution of Sericin from the Silk Cocoons of Silkworm, *Bombyx mori* (L.) For the Control of Diabetes in Brown Rat, *Rattus norvegicus* (L.).

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Abstract

The attempt was carried to study the influence of sericin from the silk cocoons of silkworm, *Bombyx mori* (L.) for the control of streptozotocin (STZ) induced diabetes in brown rat, *Rattus norvegicus* (L.). The aqueous sericin solution at the rate of 250 mg / Kg body weight and 500 mg / Kg body weight was administrated orally, daily, to the streptozotocin induced diabetic Group of experimental animals. The levels of Glucose in the normal non-diabetic experimental animals, male rats, *Rattus norvegicus* (L.) was found measured 84.05 (± 6.89) units. The levels of Glucose in the streptozotocin induced diabetic individuals in present attempt was found measured 302.66(± 23.764) units. The levels of Malondialdehyde (MDA) in serum was found reduced with increase in the dose of aqueous solution of sericin. There was a restoring tendency of insulin levels in the sericin treated individuals of streptozotocin induced diabetic group of experimental animals in the present attempt. Sericin is able to inhibit the effect of STZ on pancreatic beta cells. The sericin exhibit significant potent anti-diabetic compound.

Keywords: Sericin, Malondialdehyde (MDA), *Bombyx mori* (L.) Diabes Mellitus.

INTRODUCTION

One of the visions of the World Health Organization (WHO) is to reduce the avoidable burden due to non-communicable diseases (NCDs), (WHO, 2013) with a specific target to halt the rise in the rates of obesity and diabetes globally. Almost two thirds of the world's population with diabetes currently resides in low- and middle-income regions. South Asia is one of the epicentres of the diabetes epidemic and diabetes rates vary from 3.3% in Nepal to 10% in India (International Diabetes Federation, 2015). The so-called "south Asian" or "Asian-Indian" phenotype makes this ethnic group more susceptible to both type 2 diabetes and premature coronary artery disease, compared to white Caucasians (Mohan, *et al* , 1986; Unnikrishnan, *et al* , 2014). This phenotype is characterized by increased insulin resistance; high diabetes rates, despite lower generalized obesity; central adiposity; and dyslipidaemia, with raised serum triglycerides and low levels of high-density lipoprotein (HDL) cholesterol. While genetic factors might contribute a little to the south Asian phenotype, the current diabetes epidemic is fuelled predominantly by lifestyle, which is related to environmental factors. The two most important of these are unhealthy diet and physical inactivity. This article will therefore focus primarily on these two factors and examine how they are linked to the diabetes epidemic. It will also look at evidence and suggest how modifying these factors may help to prevent type 2 diabetes, or at least slow the rise in prevalence in this population. Rapid globalization and urbanization have led to a rapid nutrition transition. This has affected food cultures and brought about drastic changes in the diets and physical activity of populations. This is very much pronounced in countries of the WHO South-East Asia Region, which are experiencing high increases in the prevalence of diabetes. Some of the changes in diet include increased consumption of packaged and processed foods – mainly as refined carbohydrates like white rice, added sugars, edible refined oils and fats, and decreased consumption of whole grains, nuts, fruits and vegetables (Radhika, *et al* , 2009; NNMB Technical report no. 26, 2016; Mohan, *et al* , 2009; Anjana, *et al* , 2014 and Anand, *et al* , 2015).

Today, people in low- and middle-income countries opt for energy-dense foods, as they are cheaper and more easily available than the alternatives (Schmidhuber and Shetty, 2005). Physical inactivity is an independent risk factor for type 2 diabetes and current evidence suggests that adequate levels of physical activity may reduce the risk of type 2 diabetes by 27% (Lee, *et al* , 2012). Modern technical gadgets and use of motorized transport have reduced physical activity among children and young adults (Vaidya and Krettek, 2014). Ranasinghe *et al*. (2013) reported that the overall prevalence of physical inactivity among the population of India was 19–88%, followed by Pakistan (60%) and Sri Lanka (11–32%). The recent Indian Council of Medical Research– India Diabetes (ICMR–INDIAB) study also reported that levels of physical inactivity were high (≈55%) among AsianIndians (Anjana, *et al* , 2014).

The diabetes mellitus is a group of metabolic diseases characterized by the body's inability to metabolize carbohydrates, fats and proteins thereby causing chronic hyperglycemia (elevated blood sugar levels) (Tibrani, 2009). Uncontrolled disease will cause various complications of metabolism, macrovascular and microvascular disorders that lead to decrease quality and life expectancy of patients (Denik and Fuspita, 2009). Statistical reports from the International Diabetes Federation (Cavan, *et al* , 2015) reported that diabetes mellitus every year is increasing where 1 in 11 people have diabetes. The accumulation of diabetics in 2015 is 415 million, and it is estimated that by 2040 it will increase to 642 million people. The number of people with diabetes mellitus in Indonesia on average increased by 2% in each region so that the prevalence of diabetics is estimated to reach 21.3 million in 2030 (Kementrian Kesehatan Republik Indonesia, 2015).

The condition of chronic hyperglycemia caused by diabetes is associated with long-term damage, impaired function, and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels. In diabetics, the cause of damage to pancreatic beta cells can be caused by many factors. These factors include genetic factors, infection by germs, nutritional factors, diabetogenic substances, and free radicals (oxidative stress). Damage to pancreatic beta cells causes the body not to produce insulin, causing blood glucose levels to rise (hyperglycemia occurs). The condition of hyperglycaemia (Robertson, *et al* , 2003) may result in the formation of reactive oxygen species (ROS). Excessive ROS can cause oxidative stress and can aggravate the destruction of pancreatic beta cells. Oxidative stress occurs due to an imbalance between free radicals and antioxidants, when reacting with the fatty acid component of the cell membrane results in fat peroxidation which will lead to the breakdown of fatty acid chains into various toxic compounds and cause damage to the pancreatic cell membranes that produce malondialdehyde (MDA) (Mahardhian, *et al* , 2013).

The cocoon shell of the silkworm, *Bombyx mori* (L.) is sweet, warm in nature and non- toxic, and it can nourish *yin*, moisturize dryness, quench thirst and promote granulation. It can also be used to treat polydipsia, kidney consumption and cloudy urine, as well as polyphagia and emaciation Li SZ. Beijing, 1985 ; Bao XA. Tianjin, 1993). A piece of floss is bonded by two monofilament fibers comprising central silk fibroin and peripheral sericin. The silk industry originated in China and has developed for thousands of years. The majority of silk applications involve silk fibroin, which is used for clothing, while sericin, which accounts for 30% floss, is discarded. Increasing attention has been paid to the effective use of a large number of high-quality proteins, and sericin possesses many advantages for beauty, skin care, nutrition, anti-oxidation, and anti- cancer treatment (Manosroi, *et al* , 2010; Kim, *et al* , 2012; Seo, *et al* , 2011; Isobe, *et al* , 2012 ; Manosroi, *et al* , 2010). The silk cocoon soaked in water is a prescription for regulating blood glucose levels, and sericin can effectively protect islet cells, gonads and kidney (Chen, *et al* , 2010; Fu, *et al* , 2010; Fu, *et al* , 2011; Fu, *et al* , 2010; Hao, *et al* , 2013; Liu, et al , 2013). Preliminary studies by our research group have shown that sericin might improve aberrant Akt signaling, decrease heme oxygenase-1 expression in the hippocampus and cerebral cortex, and reduce the apoptosis of hippocampal neurons in diabetic rats, thus protecting the nervous system (Chen, *et al* , 2011; Chen, *et al* , 2012).

Sericin is a protein created by *Bombyx mori* (silkworms) in the production of silk (Padamwar and Pawar, 2004). Silk is a fibre spun by the mature fifth instar larvae of silkworm, *Bombyx mori* (L.). The silk is helping in production of its cocoon around the pupa of silkworm, *Bombyx mori* (L.). It consists mainly of two proteins, fibroin and sericin. Silk consists of 70–80% fibroin and 20–30% sericin. The fibroin is central core protein. The sericin is the gum like coating around the fibroin. The sericin is allowing fibroin fibres to stick to each other. The sericin is composed structurally of 18 different amino acids, and 32% serine, in most commonly, a randomized amorphous coil. When in the amorphous coil, sericin can also be easily be converted into a β-sheet conformation, via repeated moisture absorption and mechanical stretching. Using gamma ray examination, it was determined that sericin fibers are composed typically of three layers, all with fibers running in different patterns of directionality. The innermost layer, typically is composed of longitudinally running fibers, the middle layer is composed of cross fiber directional patterned fibers, and the outer layer consists of fiber directional fibers. The overall structure can also vary based on temperature, whereas the lower the temperature, there were typically more β-sheet conformations than random amorphous coils. There are also three different types of sericin, which make up the layers found on top of the fibrin. Sericin A, which is insoluble in water, is the outermost layer, and contains approximately 17% nitrogen, along with amino acids such as serine, threonine, aspartic acid, and glycine. Sericin B, composed the middle layer and is nearly the same as sericin A, but also contains tryptophan. Sericin C is the innermost layer, the layer that comes closest to and is adjacent to

fibroin. Also insoluble in water, sericin C can be separated from the fibroin via the addition of a hot, weak acid. Sericin C also contains the amino acids present in B, along with the addition of proline.

Sericin has also been used in medicine and cosmetics. Due to its elasticity and tensile strength, along with a natural affinity for keratin, sericin is primarily used in medicine for wound suturing. It also has a natural infection resistance, and is used variably due to excellent biocompatibility, and thus is used commonly as a wound coagulant as well. When used in cosmetics, sericin has been found to improve skin elasticity and several anti-aging factors, including an anti-wrinkle property. This is done by minimizing water loss from the skin. To determine this, scientists ran several experimental procedures, including a hydroxyproline assay, impedance measurements, water loss from the epidermis and scanning electron microscopy to analyze the rigidity and dryness of the skin. The presence or sericin increases hydroxyproline in the stratum corneum, which in turn, decrease skin impedance, thus increasing skin moisture. Adding in pluronic and carbopol, two other factors that can be included in sericin gels, perform the action of repairing natural moisture factors (NMF), along with minimizing water loss, and in turn, improving skin moisture (Padamwar and Pawar, 2004). The chemical composition of sericin is $C_{30}H_{40}N_{10}O_{16}$. The chemical structure of the sericin is not entirely clear or confirmed, but in a Japanese study conducted in 2007/2008 by Mayer, S. and Maric, M. (2008) suggested the chemical structure as shown Fig. 1.

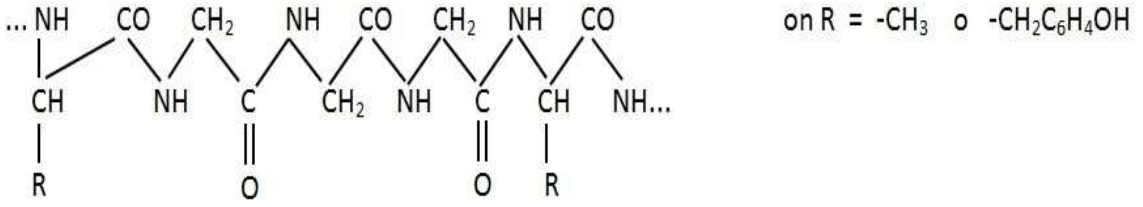

Fig. 1: The chemical structure of Sericin suggested by Mayer and Maric (2008) (Ready Source: https://en.wikipedia.org/wiki/Sericin).

The fibroin is the water-insoluble protein and sericin is water soluble. T solubility, molecular weight, and gelling properties of sericin depend exclusively on the method of isolation. The sericin has wide applications in pharmaceuticals and cosmetics such as, wound healing, bioadhesive moisturizing, antiwrinkle and antiaging. The fibroin of silk fibre of mulberry silkworm, has been recognized as a substrate for growth and adherence of cells in culture and sericin is used as abiomaterial (Khyade, 2004). This utilization is due to its antibacterial and UV resistant properties of sericin. (Vitthalrao B. Khyade, 2016). Most of silk sericin must be removed during raw silk production at the reeling mill and other stages of silk processing. At present, silk sericin is mostly discarded in silk processing waste water. If silk sericin is recovered and recycled, it can represent a significant economic and social benefit. Silk sericin is a natural macromolecular protein derived from the silkworm *Bombyx mori*. Silk sericin is useful because of its antioxidant activity. Silk sericin can be cross linked, copolymerized and blended with other macromolecular materials, especially artificial polymers, to produce materials with improved properties. The protein is also used as an improving reagent or a coating material for natural and artificial fibers, fabrics and articles. The materials modified with silk sericin and sericin composites are useful as degradable biomaterials, biomedical materials and functional membranes. The sericin treatment definitely serving to restore the original membrane integrity of the cells. The aqueous form of silk sericin, from a natural source like silkworm cocoon, serve as ideal antioxidant source and may be used to treat the cancer cells. There are some reports on lipid peroxidation through sericin, antitumor properties sericin and with

no immunogenicity. All the biomaterial related applications of silk proteins involve in vitro studies on cells prior to their implantation invivo.

According to the Control Ecological Life Support System, the Japanese Aerospace Exploration Agency, the silk protein has been used as diet. They have devised a recipe on using silk protein for astronaut food. Moreover, dried cocoon powder when used to feed poultry and fish has reported better growth rate, improved quality of egg and prolonged survival of hens (Reddy, 2009). The sericin is hydrophilic in nature and has a strong affinity for other proteins. In general, silk fiber is nontoxic to degradation, as its amino acid composition is similar to that found in humans. The high concentration of these amino acids in sericin causes chelation with different metal ions, with interpositioning of hydroxyl groups resulting in increased capacity for water retention. Furthermore, it is resistant to degradation by proteases. In the year 1998, the Japanese Association for Dietary Fiber Research stated that "food ingredients that are hardly digestible or absorbable within the human small intestine and exhibit a physiological effect that is useful for the maintenance of good health via the digestive tract" should be collectively termed as "luminacoids," which means the substances which possesses variable physiologic properties. Proteolytic enzymes play an important role in research labs from food industries to pharmaceuticals (Kato *et al* , 1998). The sericin is a scalable protein, can be manipulated to a greater extent without altering its physical and chemical properties and nutritional value (Agyei, *et al* , 2014). Various studies have reported that dietary sericin reduces the level of serum cholesterol and triglyceride in rats. The reduction in serum triglyceride also causes reduction in very low-density lipoprotein (VLDL) without affecting the serum high-density lipoprotein levels (Kato, *et al* , 2002). As high level of triglyceride and VLDL increases the chance of atherosclerosis, consumption of sericin can be helpful in the prevention of atherosclerosis. Sericin also exerts an inhibitory effect on the accumulation of lipids in the liver and causes decreased release of triglycerides to the serum. The free fatty acids in the serum are reduced with sericin intake, without affecting the activity of carnitine palmitoyltransferase I (CPT I) which is a rate limiting enzyme for the oxidation of fatty acids. The suppressive effect of sericin on free fatty acids is associated with increased peripheral glucose uptake, bringing about a better glucose tolerance. It has been reported that a dietary addition of two percent sericin, significantly reduces the oxidative stress in rats. Recently, it has been found that sericin increases the antioxidant activity in rats by inhibiting tyrosinase (Martin, *et al*, 2005; Agyei, *et al* , 2014). The sericin has an anti-constipative effects, as it causes increased excretion of fecal nitrogen, thereby causing increased evacuation in rats (Kato, *et al* , 1998; Kato, *et al* , 2002). Furthermore, sericin increases intestinal absorption of various trace elements such as zinc, magnesium, and iron, and the resultant is increase in bioavailability of these substances (Sasaki, *et al* , 2008). Sericin is also an anti-frosting agent, and coating of it if applied on raw fruits and vegetables can prevent them from freezing (Rangi, 2015).

There are reports on the antioxidant influence of sericin of silk of the cocoons of the mulby silkworm, *Bombyx mori* (L.) and non-mulberry, tropical tasar silkworm *Antheraea mylitta* (L.), in skin fibroblast cell line, exposed to hydrogen peroxide for 24 Hours (Vitthalrao B. Khyade, 2016; Sharad G. Jagtap and Vitthalrao B. Khyade, 2016). The study carried out by 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. Rare reports on sericin to be utilized in treating the diabetes made to plan the present study on utilization of aqueous solution of sericin from the silk cocoons of silkworm, *Bombyx mori* (L.) for the control of diabetes in brown Rat, *Rattus norvegicus* (L.).

MATERIAL AND METHODS

The attempt was carried through the steps like: Rearing of experimental animals; Induction of diabetes through streptozotocin; Separation and Isolation of Sericin from cocoons; Grouping the experimental animals; Preparation of the Samples For Biochemical Assays; Serum Collection; Bioassay of Malondialdehyde Levels in Serum; Estimation of insulin level; Histological Analysis of Pancreatic Tissues and Statistical analysis.

(A). Rearing of Experimental Animals:

Six week old male rats (*Rattus norvegicus* L.) were obtained from Department of Zoology, University of Pune. The rats were housed in four cages and maintained at 28 degree Celcious and subjected to a 09:15 hours light – dark cycle (Lights on 8.00 a.m. to 5.00 p.m.).The rats in cages were acclimatized for one week before the experimental use. The rats were feed a commercial stock diet and deionized water. The rats were maintained in laboratory through the standard methods. The body weight was measured every week. For the present attempt, permission was issued by the ethical committee of Animal Welfare and Use Committee, Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar **(**Tal. Baramati Dist. Pune – 413115 India).

(B). Induction of Diabetes Through Streptozotocin:

Streptozotocin (STZ) is a synthetic antineoplastic agent that is classifically an anti-tumor antibiotic and chemically is related to other nitrosureas used in cancer chemotherapy. Streptozotocin sterile powders are provided and prepared as a chemotherapy agent. Each vial of sterilized Streptozotocin powder contains 1 gr. of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2-[[(methylnitrosoamino)-carbonyl] amino]-D-glucopyranose and 200 mg. citric acid. Streptozotocin was supplied by Sigma Chemical Company. Streptozotocin is available for intravenous use as a dry-frozen, pale yellow, sterilized product. Pure Streptozotocin has alkaline pH. When it is dissolved inside the vial in distilled water as instructed, the pH in the solution inside the vial will be 3.5-4.5 because of the presence of citric acid. This material was prepared in 1-gr vials and kept in cold store and refrigerator temperature (2-8 °C) away from light. The rats weighting 250-300 grams (75-90 days old) were used for inducing diabetes. A freshly-prepared solution of streptozotocin (STZ, 70 mg/kg/bw) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally to rats that had fasted overnight (Kesari *et al*., 2007). Streptozotocin induces diabetes within 3 days by destroying the beta cells (Karunanayake, *et al* , 1975). Diabetic animals and non-diabetic control group were kept in metabolic cages individually and separately and under feeding and metabolism control. Glucose in the blood of diabetic rats exceeded that of the non-diabetic control ones. The diabetic rats have glucose level above 200 mg/dL. Food consumption was measured in terms of (gr.), water consumption was measured in terms of (ml) and urine volume was measured in terms of (ml) on a daily basis while every 2- 4 weeks in 80 days the levels of C-peptide, insulin and glucose in blood serum were also measured, so that chemical diabetes was verified in rats injected with Streptozotocin (Bhuyan, *et al* , 1974).

One week later, blood samples were collected from the orbital sinus, and rats with fasting blood glucose (FBG) levels above 200 mg/dL (11.1 mmol/L) were selected for the experimental protocol. During a 21-day period of treatment, normal and STZ-treated rats were fed with 40 g/day of pellet food **(**Hindustan Animal Feeds, Behind Gokulnagar Octroi Check Post, Near Vijaynagar Railway Crossing, Jamnagar – 361004 Gujarat INDIA).

(C). Separation and Isolation of Sericin from cocoons: The sericin was separated and isolated from the cocoons of mulberry silkworm *Bombyx mori* (L.) through the use of standard protocol described by Sofia, *et al* (2001). The cocoons were procured from Sericulture Unit, Malegaon Sheti Farm, Agricultural Development Trust Baramati, Shardanagar, (Malegaon Khurd) Post Box No - 35, Baramati, Pune 413 115, Maharashtra, India. The cocoons were processed for separation of the pupa and the shell. The cocoon shells were the cut into smaller pieces. The cocoon shell pieces were boiled in the solution of 0.02 M Na_2CO_3. This boiling was carried out for half an hour. The supernatant of resulted solution was decanted out and was dialyzed to obtain sericin. The protein solution was dialyzed against several changes of Milli Q water (ultrapure water of Type 1). The crude extracts of sericin (along with all fractions) were used for further experimentations. Further, 8 % SDSPAGE was carried out to confirm the presence of proteins.

(D). Grouping the experimental animals:
Forty healthy adult, eight weeks old , weighing between 150 and 250 g male rats, *Rattus norvegicus* L. were used in this experiment. Rats were divided into four groups (15 rats in each groups). After diabetic state was confirmed, the rats, the experimental animals were used for the studies on Sericin treatment. They were divided into in four experimental groups (n=15 animals/ group):

Group – (I) Untreated Control Group. (treated with vehicle water): Received 100 mL of water a day.
Group – (II) Streptozotocin Induced Diabetic Group: Received 100 mL of water a day.
Group – (III) Sericin Treated Streptozotocin Induced Diabetic Group – 1 : Treated with water solution of Sericin at the rate of 250 mg / Kg body Weight.
Group – (IV) Sericin Treated Streptozotocin Induced Diabetic Group – 2 : Treated with water solution of Sericin at the rate of 500 mg / Kg body Weight).
The diabetic group rats were allowed to drink a 5% glucose solution overnight to overcome the drug-induced hypoglycemia. The rats of the control and diabetic groups were fed without any supplements except vehicle water, which was used for dissolving the Sericin. The aqueous Sericin solution at the rate of 250 mg / Kg body weight was administrated orally, daily, to the rats of "Sericin Treated Streptozotocin Induced Diabetic Group – 1" . The aqueous Sericin solution at the rate of 500 mg / Kg body weight was administrated orally, daily, to the rats of "Sericin Treated Streptozotocin Induced Diabetic Group – 2" .

(E). Preparation of the Samples For Biochemical Assays:

Blood sugar levels were measured before and after 48 h of STZ induction. After 48 h of STZ induction, the rats whose blood glucose levels were ≥200 mg/ dL were considered as diabetic. Plasma glucose levels and the body weights of the animals were measured weekly for the duration of the study. The rats were anesthetized by an intramuscular injection of 50 mg/kg of ketamine, and blood was taken by puncturing the heart ventricle at the end of the experiment. Blood samples were centrifuged at 3000 rpm for 20 min and the plasma was separated. Red blood cells that remained on the bottom of the tubes were washed with a phosphate buffer, pH 7.4, and the samples were then kept at –20 °C until they were analyzed.

(F). Serum Collection: Freshly collected blood was allowed for coagulation. After blood coagulated, about 2 hours after collection, blood was centrifuged at 3000 rpm for 15 min, in order to collect blood serum. Serum was moved into Eppendorf tube, and then serum was centrifuged at 1000 rpm for 10 minutes. Serum samples were moved into new Eppendorf tube and stored in -20oC freezer until malondialdehyde levels determination.

(G). Bioassay of Malondialdehyde (MDA) Levels in Serum:
Malondialdehyde levels were determined using *thiobarbituric acid* (TBA). The bioassay was carried out through the method explained by Ameen Turki and Moayad Naji Majeed (2011).The Millon reagent levels measurement was performed by adding 100 µL serum with 550 µL distilled water, 100 µL TCA 20%, 250 µL HCl 1N, 100 µL Na-Thiobarbiturate then incubated for 30 minutes at 100°C. After incubation, sample reading was performed using spectrophotometer UV-Vis at the wavelength of 533 nm. The levels of MDA was expressed in the unit: nmol/g protein.

(H). Estimation of insulin level: The concentration of insulin in serum samples was estimated using Enzyme-Linked Immunoabsorbent Assay (ELISA) method using insulin kit from Syntron Bioresearch (USA). The sample used was non-haemolysed serum. Following a standard procedure, a sample of the standard curve was plotted and insulin concentrations in the samples were determined by interpolation from the standard curve (Calabrese, *et al* , 1981; Widajaja, *et al* , 1997).

(I). Histological Analysis of Pancreatic Tissues:

Pancreas was fixed in paraformaldehyde solution and was dehydrated with a gradual ethanol series, and then were embedded in paraffin to bring out ultrathin sections of the pancreas. Furthermore, the ultrathin sections were stained with Hematoxylin-Eosin. First, the ultrathin sections were deparaffinized with xylol and rehydrated with a gradual ethanol series (absolute, 95, 90, 80 and 70%) respectively for 5 minutes. Then those were soaked in the distilled water for 5 minutes. Furthermore, the ultrathin sections were dyed with hematoxylin and were incubated for 10 minutes to obtain the best color results. Then the ultrathin sections were washed with flowing water for 30 minutes and rinsed with distilled water. Next, the ultrathin sections were dyed with eosin with alcohol for 5 minutes. The last steps were dehydrated using a gradual series of ethanol (80%, 90%, 95%, and absolute) and cleared with xylol then dried. The dried and ultrathin stained sections were mounted with Entellan and were observed under a microscope with a magnification of 400 times.

(J). Statistical Analysis:
The data obtained were analyzed by using Shapiro-Wilk statistic and homogeneity in order to determine the normality of data distribution. Effects of treatment on parameters of total malondialdehyde level were analyzed using ANOVA which was completed by Tukey test with 95% confidence level to know the difference between treatments. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) 23.0 software.

RESULT AND DISCUSSION

The results on Utilization of Aqueous Solution of Sericin from the Silk Cocoons of Silkworm, *Bombyx mori* (L.) For the Control of Diabetes in Brown Rat, *Rattus norvegicus* (L.) are summarized in Table – 1 ; Figure: 2 – 5 and explained parameter wise: Glucose Levels in Serum; Malondialdehyde Levels in Serum; Estimation of insulin level and Histological Analysis of Pancreatic Tissues.

(I). Glucose Levels in Serum:
The levels of Glucose in the normal non-diabetic experimental animals, male rats, *Rattus norvegicus* (L.) was found measured 84.05 (± 6.89) units. The levels of Glucose in the streptozotocin induced diabetic individuals in present attempt was found measured 302.66(± 23.764) units. normal non-diabetic experimental animals, male rats, *Rattus norvegicus* (L.) was found measured 84.05 (± 6.89) units.

Treating the streptozotocin induced diabetic individuals with aqueous solution of sericin at the dose of 250 mg / Kg body weight was found effected into significant increase in the glucose level, measuring 142.53 (±26.081) units. Treating the streptozotocin induced diabetic individuals with aqueous solution of sericin at the dose of 500 mg / Kg body weight was found effected into glucose level measured 108.24 (±11.786) units. The glucose level was found reduced with increase in the dose of aqueous solution of sericin.

(II). Malondialdehyde (MDA) Levels in Serum:
The levels of Malondialdehyde (MDA) in serum in the normal non-diabetic experimental animals, male rats, *Rattus norvegicus* (L.) was found measured 0.165 (± 0.048) units. The levels of Malondialdehyde (MDA) in serum in the streptozotocin induced diabetic individuals in present attempt was found measured 0.234 (±0.07) units. Increased levels of MDA is the distinguishing feature of stress caused through diabetes.

Treating the streptozotocin induced diabetic individuals with aqueous solution of sericin at the dose of 250 mg / Kg body weight was found effected into MDA level measured 0.169 (± 0.042) units. Treating the streptozotocin induced diabetic individuals with aqueous solution of sericin at the dose of 500 mg / Kg body weight was found effected into MDA level measured 0.076 (±0.008) units. The MDA level was found reduced with increase in the dose of aqueous solution of sericin. Statistical test results showed that there were significant differences ($P<0.05$) between MDA levels in diabetic individuals treated 500 mg / Kg body weight than that of sericin treatment at the dose of 250 mg / Kg body weight. This observation exert to label the sericin as antioxidant, especially as a hydroxyl radical scavenger.

(III). Insulin level :
The levels of insulin in the normal non-diabetic experimental animals, male rats, *Rattus norvegicus* (L.) was found measured 9.951(±1.183) units. The levels of insulin in the streptozotocin induced diabetic individuals in present attempt was found measured 7.683 (±0.941) units. The decrease of insulin content in experimental animal serum was decreased by 22.79%. The decrease in insulin levels in the blood is due to the presence of diabetogenic agents (MLD-SZT) that are induced in animal experiments so that pancreatic beta cells are damaged over time. Streptozotocin can affect pancreatic beta cells because it produces free radicals in the form of hydrogen peroxide (H2O2) and superoxide anions (O2 -).
Treating the streptozotocin induced diabetic individuals with aqueous solution of sericin at the dose of 250 mg / Kg body weight was found effected into insulin level measured 8.714 (± 1.359) units. Treating the streptozotocin induced diabetic individuals with aqueous solution of sericin at the dose of 500 mg /

Kg body weight was found effected into insulin level measured 89.426 (± 1.421) units. There was a restoring tendency of insulin levels in the sericin treated individuals of streptozotocin induced diabetic group of experimental animals in the present attempt. Sericin was able to inhibit the effect of STZ on pancreatic beta cells, although only slightly.

(IV). Histological Analysis of Pancreatic Tissues:

Changes in pancreatic endocrine cells was observed through pancreatic histopathologic preparations stained with hematoxylin Eosin (HE) staining. Figure - 5 shows a change of pancreatic histology in the streptozotocin-induced group of group of rats, *Rattus norvegicus* (L.). This is in comparison with the group treatment group of individuals with aqueous solution of sericin at the dose of 250 mg / Kg body weight and treatment group of 500 mg/kg bw sericin. The histological changes of the pancreas can be seen from smaller endocrine cells even begin to disappear so that only the empty cytoplasm is visible. In addition, the size of the island of Langerhans on preparations of group B has a smaller size compared to the island of Langerhans on preparations of groups A, C and D. In preparatory group B is also seen the morphology of the pancreas organ that there are gaps or cavities in both endocrine areas (the island Langerhans) as well as in the exocrine region (acinar cells) that indicate a degeneration process. Langerhans Island in negative controlled animals showed more pancreatic cells, especially pancreatic beta cells than in positive controlled animals, where there was no widening of cavities (intercellular space) on Langerhans island. The widening of the cavity in the pancreatic histopathological picture in the positive control group was due to the severe necrosis that the cell nucleus suffered from death resulting in a shift.

According to Wolf (1993), mechanisms involved in the elevation of oxidative stress in diabetic individuals are mostly auto oxidative glycosylation, nonenzymatic glycation, metabolic stress resulting from changes in energy metabolism, changes in the level of inflammatory mediators and the status of antioxidant defense systems. The components of total antioxidant such as vitamin E and glutathione, cause removal of free radicals from the body. The reducing tendency in the activity of malonaldihyde (MDA) in the sericin treated diabetic rats observed in this attempt may reduce the total antioxidant response (Aragno, *et al* , 1997). It is probable that the sericin directly activates the enzyme responsible for reduction in oxidative stress. It may also increase expression of such enzymes. According to Bastar, *et al* (9198), the antioxidants enter to the circulation via passing through small intestine, where they do perform their biological functions as antioxidant. On this line, present attempt is concluding that, the sericin, the antioxidant potential may enter to the circulation via passing through small intestine, where it have to express through it's biological functions as antioxidant.

Administration of streptozotocin (STZ) (70 mg kg^{-1} b.wt. I.P.) induced hyperglycemia **(**blood glucose level ≥200 mg dL^{-1}) in the rat, *Rattus norvegicus* (L.). Diabetes induced by STZ was characterized by apoptosis of cells of pancreas, attenuation of gene expression of insulin and reduced synthesis of insulin. The cells of pancreas normally maintain blood glucose concentrations within a narrow range by modulating their insulin secretion rate in response to the blood glucose concentration apoptosis of pancreatic cells is believed to be the primary factor which ultimately results in hyperglycemia (Patel *et al*., 2006).

According to Yavorska (2012), the viscous dietary fibres dampen the rise in blood glucose levels following food intake by delaying gastric emptying and slowing the absorption of nutrients in the small intestine. Whether one of these mechanisms or both of them enable sericin's effect on glycemic response attenuation was unclear.

The mechanism of decreased insulin secretion in diabetic individuals could be attributed to the resultant hyperglycemia that induced abnormalities in insulin action and secretion (Rossetti *et al*., 1987; Evans *et al*., 2003). Hyperglycemia is also associated with the consequences of hyperinsulinemia, insulin resistance, and glucose intolerance in diabetes (Kaur *et al*., 2002). The hypoglycemic effect of Sericin in the present attempt may be related to the ability of its fiber to produce high viscosity at low concentration in the gut lumen The fibrous compounds deserve ability of retaining water and have the property of forming colloidal gels. This decreases the association of food with the intestinal mucosa and the enzymatic digestion rate, consequently decreasing the intestinal absorption of monosaccharides and disaccharides (Wilson *et al*., 1998) by delaying gastric emptying or by interaction with digestive enzymes of the intestine (Nandini *et al*., 2003).

Last but not least, the histological changes in the pancrease of the experimental animals of present attempt. According to Julie and Jurenka (2009), the changes in cells caused by substances that have a cytotoxic effect are the reduction of the Langerhans islands, the reduction in the number of β cells and degranulation, the vacuolization of these cells. In people with diabetes mellitus, some β cells show complete degranulation and an empty cytoplasm. The results of observations of pancreatic histopathologic preparations from experimental rats treated with sericin exhibited better Langerhans island than the pancreatic group of Positive Control (B) rat (Figure 5). Changes that occur include the number of β cells are more, β cells spread throughout the island Langerhans, larger island size Langerhans and cavities in the endocrine region of the pancreas fewer than the pancreas in the KP group. This change shows the endocrine cells that begin to regenerate into normal shape.

This is due to the treating the diabetic individuals with aqueous solution of sericin. The sericin may be responsible for inhibition of the damage of β cells. Antioxidants are substances that the body needs to neutralize free radicals and prevent damage caused by free radicals to cells. Antioxidants stabilize free radicals by supplementing electron deficiencies possessing free radicals and inhibiting the occurrence of chain reactions from the free radical formation which can cause oxidative stress. Next step on utilization sericin for treating the diabetes should be screening the sericin from the cocoons of different types silkworms.

CONCLUSION

Treating the streptozotocin induced diabetic individuals of rat, *Rattus norvegicus* (L.) with aqueous solution of sericin at the dose of 250 mg / Kg body weight and 500 mg / Kg body weight was found effected into significant increase in the glucose level. The glucose level was found reduced with increase in the dose of aqueous solution of sericin. Critical analysis of the results that there were significant differences ($P<0.05$) between MDA levels in diabetic individuals treated 500 mg / Kg body weight than that of sericin treatment at the dose of 250 mg / Kg body weight. This observation exert to label the sericin as antioxidant, especially as a hydroxyl radical scavenger. There was a restoring tendency of insulin levels in the sericin treated individuals of streptozotocin induced diabetic group of experimental animals in the present attempt. Sericin was able to inhibit the effect of STZ on pancreatic beta cells, although only slightly. The histological changes of the pancreas can be seen from smaller endocrine cells even begin to disappear so that only the empty cytoplasm is visible. Sericin deserve anti-oxidative potential and exert positive influence to over come the damage caused by diabetes.

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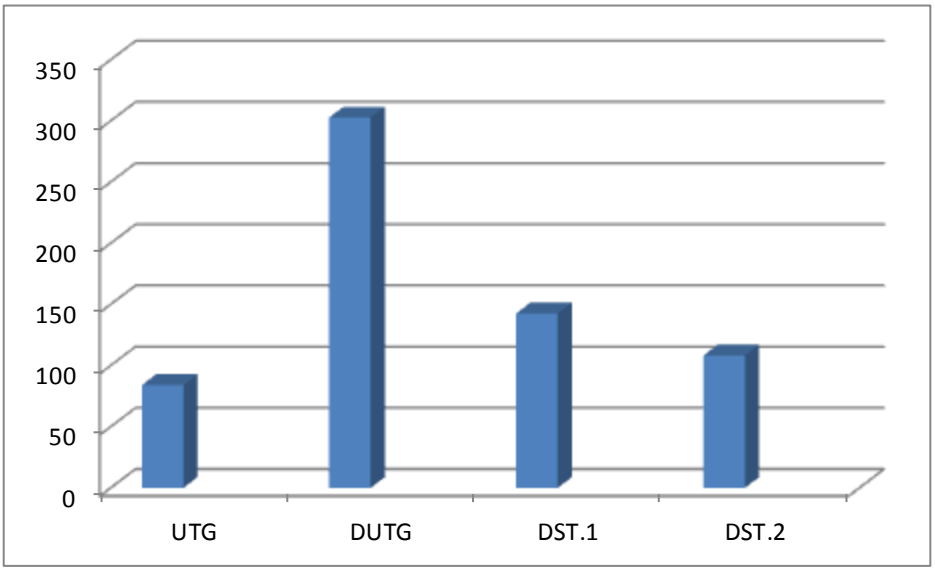
Table – 1: Effect of Sericin administration on the glucose level; malondialdehyde (MDA) and insulin level in the blood of brown rat *Rattus norvegicus* (L.).

Parameter Group	Blood Glucose	MDA	Insulin
Untreated Control	84.05 (± 6.89)	0.165 (± 0.048)	9.951 (±1.183)
Diabetic Untreated	302.66** (± 23.764)	0.234* (±0.07)	7.683* (±0.941)
Diabetic Group Treated with Sericin (250 mg / Kg body Weight).	142.53** (±26.081)	0.169*** (± 0.042)	8.714*** (± 1.359)
Diabetic Group Treated with Sericin (500 mg / Kg body Weight).	108.24*** (±11.786)	0.076*** (±0.008)	9.426*** (± 1.421)

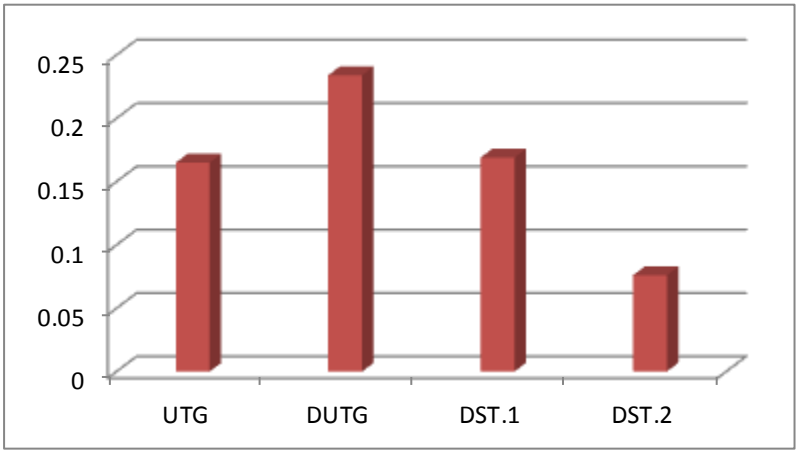
- Unit for Plasma Glucose Concentration: (mg/dL).
- Unit for MDA: (nmol/g protein).
- Unit for Plasma Insulin Concentration: (mIu/l).
- Each figure is the mean of three replications.
- Figures in parentheses with ± sign are the standard deviations.
- *: P< 0.001
- **: P< 0.05 compared to the control.
- *: P < 0.05 compared to the STZ-diabetic group.

Figure – 2: Effect of Sericin administration on the glucose level in the blood of brown rat *Rattus norvegicus* (L.).



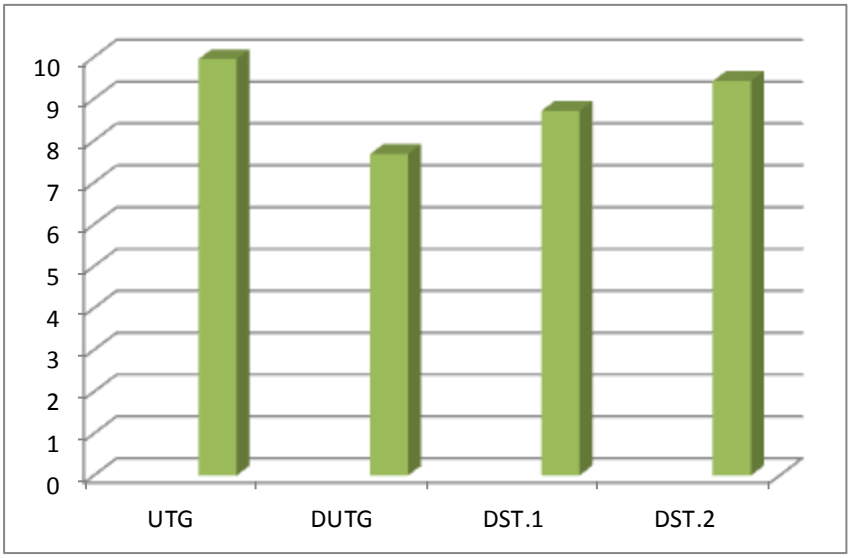
UTG: Untreated Normal Group; DUTG: Diabetic Untreated Group; DST.1: Diabetic Group Treated with Sericin (250 mg / Kg body Weight) and DST.2: Diabetic Group Treated with Sericin (500 mg / Kg body Weight).

Figure – 3: Effect of Sericin administration on the Malondialdehyde (*MDA*) level in the blood of brown rat *Rattus norvegicus* (L.).



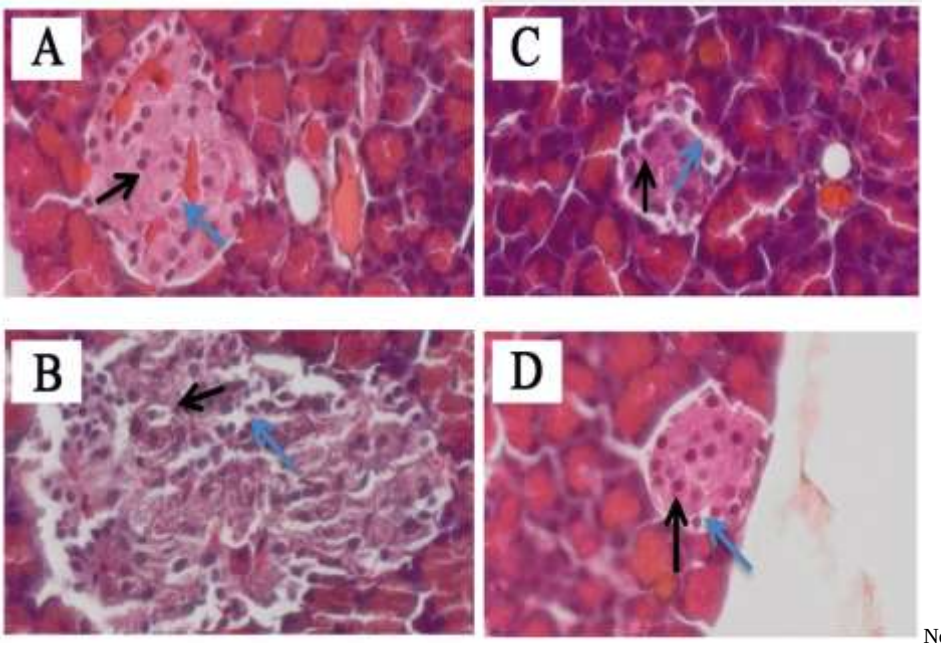
UTG: Untreated Normal Group; DUTG: Diabetic Untreated Group; DST.1: Diabetic Group Treated with Sericin (250 mg / Kg body Weight) and DST.2: Diabetic Group Treated with Sericin (500 mg / Kg body Weight).

Figure – 4: Effect of Sericin administration on the Insulin level in the blood of brown rat *Rattus norvegicus* (L.).



UTG: Untreated Normal Group; DUTG: Diabetic Untreated Group; DST.1: Diabetic Group Treated with Sericin (250 mg / Kg body Weight) and DST.2: Diabetic Group Treated with Sericin (500 mg / Kg body Weight).

Figure – 5 : Effect of Sericin administration on the Histological Structure of Pancrease of brown rat *Rattus norvegicus* (L.).



Normal Pancreatic Histology (A) magnified 400 times (Control rat (A), diabetic rat (B) therapeutic rat treated with Sericin 250 mg/kg bw (C), and 500 mg/kg bw (D). (Cell nucleus (blue arrow), widening the cavities (cellular spaces) (black arrow).