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RESEARCH ARTICLE

GASTROPROTECTIVE EFFECT OF CRUDE SERICIN POWDER, AGAINST ASPIRIN INDUCED GASTRIC MUCOSAL INJURIES IN RATS

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Abstract

Crude Sericin Powder, derived from the silk gland of silkworm (*Bombyx mori*) has many biomedical applications due to its antioxidant properties. In this present study , to evaluate the gastro-protective consequences of hydroxyl amino acid rich sericin solution on aspirin induced gastric mucosal injuries in albino rat models were tested following Institutional Ethical committee rules and regulations .In our experiments, 36 male Holtzman strain adult rats were considered and divided into Control group, Crude sericin treated group, Crude Sericin Pre-treated control group, Crude Sericin Pre-treated Aspirin treated group, Omeprazole treated control group and Omeprazole Pre-treated experiment group. AST, ALT and ALP, SOD, CAT, LPO, and GSH were measured of all groups. Crude Sericin Pre-treated group shows increase in UI, GSH, ALT ALP and AST activities, whereas it shows decrease in CAT and SOD activities.

Introduction:-

An ulcer is discontinuous or breaks in the body membrane that impedes normal functions of the affected organ. According to Robbins pathology, "ulcer is the breach of the continuity of skin, epithelium or mucous membrane caused by blowing out of inflamed necrotic tissue."

Various types of ulcers have been found in the human body. Amongst which are- Peptic ulcer (inflammation inside gastro intestinal mucosa), Gastro duodenal ulcer (inflammation found in inside the GI tract), Genital ulcer(an ulcer in genital areas),Diabetic foot ulcer(complications found in diabetic foot), Pressure ulcer (also known as bed sore) Venous ulcer(an open sore in the veins due to improper functioning of valves) , Anal fissure (ulcer in anus or within

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rectum),Stress ulcer(an ulcer within stomach and proximal duodenum due to stress), Corneal ulcer(when cornea get affected), and Mouth Ulcer (inflammatory condition inside the mouth).

Gastroduodenal ulcer or peptic ulcer have been mostly observed ulcers in humans.

Gastric ulcer developed due to the reasons like H. pylori bacteria occurrence, NSAIDS drugs like aspirin, Gastric factor, Smoking, Alcohol consumption, Irregular diet habit, Oxidative stress.

Most people will be prescribed a medication called proton pump inhibitor to reduce the amount of acid their stomach produces and it allows the answer to heal naturally. Some PPI are Omeprazole, Lansoprazole, Pantoprazole, Rabeprazole, Esomeprazole etc

If an *H. pylori* infection is responsible for ulcers, Triple Drug Therapy (PPI+2 AMA) will be used like some PG analogue are also help in treating gastric ulcer like Misoprostol, Alprostadil etc

H2 receptor Antagonist use in gastric ulcer treatment like Cimetidine, Ranitidine, Famotidine, Nizatidine, Roxatidine etc.

Crude sericin powder, derived from the silk gland of silkworm Bombyx Mori, belonging to family Bombycidae, order lepidoptera, class Insecta, phylum Arthropoda and kingdom Animalia, has many biomedical applications. A few studies show that the immune system activation from the silk protein and histologically the hypersensitivity reactions were attributed to the sericin. The use of Sericin protein in cosmetic formulation such as creams and shampoos lead to an increased hydration, elasticity cleaning with less irritation and also prevents nails from chapping and brittleness. Several studies provide evidence of the healing properties of Sericin sins it operates in stimulating the migration proliferation and production of collagen. Sasaki et al, found the antitumor effect of Sericin on colon tumorigenesis in animal models. Sericin supplement used on optimum doses can reduce the problem of constipation .The Sericin residue of Sericin protein extracted from cocoons of bombyx Mori using chlorosulfonic acid. Obesity, a worldwide epidemic, is characterised by an exclusive body fat increase accompanied by a number of comorbidities. In this regard various starting points to the promising effect of Sericin on lipid metabolism and obesity. Epithelial and connective tissue repair on the tissue engineering normally uses bio material that the specific structure of that issue replaces and must be capable in turn of being replaced in time via ingress of new cells. Teramoto et al show that Sericin hydrogel is a natural biomaterial. Sericin can be used for drug delivery due to its chemical reactivity that enables the easy binding of other molecules and pH responsiveness allowing the fabrication of small materials. Dietary antioxidants have been of great interest especially due to the findings on the effect of free radicals in the body which can have serious consequences if their products are not neutralised by an efficient antioxidant system. In this sense, studies have shown the antioxidant properties of Sericin. Kato et al, showed for the first time in in vitro study that Sericin inhibits rapid peroxidation.

Cocoons of *Bombyx mori*i can provide natural pigments typically flavonoids and carotenoids that accumulated in the Sericin layer. These pigments are known for their biological properties as antioxidants and anti-tyrosinase.

Sericin powder was found to be a potential antioxidant drug which reduces the blood sugar level in alloxan induced diabetic rats. Several elements like polyphenols and flavonoids have been isolated from Sericin. These elements reported to you have a role in protection against gastric mucosal damage. It has been found SMSP or steam and freeze-dried mature silkworm larval powder source gastroprotective effect against ethanol induced gastric mucosal injuries in rats. Stress, non-steroidal anti-inflammatory drugs (NSAID) and helicobacter pylori are the most common causes of this ulceration.

Repeated use of this drug mainly causes gastrointestinal bleeding and large doses can prove a host of reactions including ulcer vomiting diarrhoea vertigo and hallucinations. Chronic NSAID users have a 42-44% risk of developing a symptomatic ulcer with gastrointestinal bleeding.

This present study is done in order to evaluate the gastroprotective effect of crude sericin powder in aspirin induced gastric mucosal injury in rats.

Material and Method:-

Duration of The Project:
From January 2019 to December 2019

Collection of Silkworms for sericin preparation:
Silk worm *Bombyx mori* were collected from Department of Sericulture, Krishnath College, Berhampore, Murshidabad.

Preparation of crude sericin Powder:
- Fifth instar larvae of *Bombyx mori* were collected.
- Middle Silk glands (MSG) were removed and washed with deionized water.
- The glands were then squeezed into a beaker containing deionized water and stirred.
- The resultant solution was then centrifuged and supernatant.
- Protein concentration was determined.
- Sericin thus obtained was electrophoresed under reducing conditions.

Chemicals:
Aspirin and Omeprazole were collected.

Maintenance of Animals:
In the following research work, 36 male Holtzman Strain albino adult rats of weight 250-300g and of nearly120 days old were chosen.Inthe following studies, 36 Holzman strain albino adult male rat of weight 250-300gm and 120 days of age approximately were chosen. According to the rules and regulations of Institutional Ethical Committee, the standard lab conditions the animals were maintained. And water was freely provided excluding at the time of testing. Animals were arbitrarily grouped as Control group, Aspirin treated Experiment Group, Sericin treated Control Group, Sericin pre-treated Aspirin treated experiment group, Omeprazole-treated Control Group and Omeprazole pre-treated Aspirin treated Experiment Group. All the animals were managed for the experiment.

Biochemical Estimations:
Collection of Serum:
The blood was collected and by centrifuged serum was separated.

Estimation of SOD (Super Dismutase):
The tissue samples homogenised with 5 mL of ice-cold 0.1 M phosphate buffer (pH-7.4). The homogenates were then centrifuged. Then, the sample was mixed with Trehalose- 6, 6 dibehenate (TDB). Reaction was started by the addition of nicotinamide adenine dinucleotide phosphate (NADPH). Then ethylenediaminetetraacetic acid manganese chloride (EDTA-MnCl2) mixture was added to it. Thereafter, spectrophotometric readings were recorded. After recording of the spectrophotometric readings mercaptoethanol was added to this mixture and againspectrophotometric readings were recorded

Estimation of ALP, AST and ALT:
According to the method of Reitman and Frankel alkaline phosphatase (ALP) activity was measured. According to the method of Kind and King, AST and ALT activities were measured.

Estimation of LPO (Lipid Peroxidation):
According to the method of Roy *et al*. and Hazra *et al*. LPO was estimated. With 0.1 M phosphate buffer (pH-7.4) tissue samples were homogenised and then centrifuged. After that sample with TDB was incubated. Later on trichloroacetic acid (TCA) was mixed, vortexed and the absorbance was read. After recording the spectrophotometric reading, mercaptoethanol was mixed withsample and read the absorbance.

Estimation of Catalase Activity (CAT):
Cohen *et al*. Catalase (CAT) activity was estimated with the help of the method of Roy *et al*. With 0.1 M phosphate buffer (pH-7.4) tissue samples were homogenised. Then ,homogenates were centrifuged. Then blended with the addition 0.1 M phosphate buffer and allowed to stand in cold condition with infrequent shaking. The shaking process was repeated. Then sample was mixed H2O2. The rate of decomposition of H2O2 was measured spectrophotometrically. The activity of CAT was calculated as % inhibition unit.

Estimation of Reduced Glutathione (GSH):
By using the method of Ellman Reduced glutathione (GSH) was measured. With TCA equal quantity of homogenate was mixed and centrifuged in order to get the proteins. Then phosphate buffer (pH- 8.4), 5,- 5-dithiobis- (2-nitrobenzoic acid) and double-distilled water were added to this supernatant . The mixture was vortexed and the absorbance was read. As µg/g of tissue the concentration of GSH was expressed.

Statistical Analysis:
Followed by multiple comparison *t*-test, which was used for statistical evaluation of the data, the data were expressed as mean ±SEM and were analysed statistically using one–way analysis of variance. Two–tailed Student's *t-tests* were performed to determine the level of significance between the means. Difference below the probability level 0.05 was considered statistically significant.

Result:-

After completion of the experiment, the AST, ALT, ALP, SOD and CAT activities, GSH and LPO levels were estimated and both ulcer index (UI) and mucosal thickness (MT) were estimated.The control group, Crude sericin powder solution - treated control group and omeprazole- treated control group showed the lowest (zero) UI. There was a sharp decline (P<0.001) in mean ulcer index both in the Crude sericin powder solution - pretreated aspirin-treated experimental group and omeprazole- pretreated aspirin– treated experimental group as compared to the aspirin-treated experimental group. The MT was significantly (P<0.001) increased in Crude sericin powder solution - treated control group compared to the control group. Crude sericin powder solution significantly (P<0.001) increased MT in Crude sericin powder solution - pretreated aspirin- treated experimental group compared to the aspirin- treated experimental group. There was a sharp rise (P<0.001) in MT in the omeprazole- treated control group compared to the control group. The MT was significantly (P<0.001) increased in omeprazole– treated control group in comparison to Crude sericin powder solution - treated control group. The MT was significantly (P<0.001) increased in the omeprazole- pretreated aspirin- treated experimental group when compared with the aspirin-treated experimental group. The results are shown in table-1

Effect of Sericulture product (SP) or Crude sericin powder solution on Ulcer Index(UI) and Mucosal Thickness(MT) of stomach and duodenum tissues:

Table1:-

Group	Ulcer Index (UI)		Mucosal Thickness (MT)	
	Stomach	Duodenum	Stomach	Duodenum
Control group	00	00	565+-0.09	742.61+-0.14
Aspirin treated	41.33+-0.08	32.23+-0.02	266+-0.03***	478+-12***
SP treated control group	00	00	663.14+-0.07*	1101.13+-0.25*
SP + Aspirin treated experimental group	1.19+-0.03#	1.75+-0.05#	602.45+-0.06**	762.16+-0.07**
Omeprazole + control group	00	00	698.25+-0.06*	1178+-0.11*
Omeprazole + Aspirin treated group	1.09+-0.02#	1.16+-0.02#	624.44+-0.07**	779+-0.09**

SP: Sericulture Product, values are mean ± SEM , n=6 , Data were analysed statistically using one-way analysis of variance test followed by multiple comparison t-test, * P<0.001 when compared with control group, ** P<0.001 when compared with Aspirin-treated group,*** P<0.001 when compared with other mentioned Groups, #P<0.001 when compared with Aspirin- treated group.

There was a sharp rise (P<0.001) in AST, ALT and ALP activities in the aspirin- treated experimental group as compared to the control group. The AST, ALT and ALP activities were significantly (P<0.001) decreased in Crude sericin powder solution- treated control group compared to the control group. Crude sericin significantly (P<0.001) decreased AST, ALT and ALP activities in Crude sericin powder solution -pretreated aspirin– treated experimental group compared with the aspirin- treated experimental group. There was a sharp decline (P<0.001) in AST, ALT and ALP activities in the omeprazole- treated control group compared to the control group. The AST, ALT and ALP activities were significantly (P<0.001) decreased in the omeprazole- treated control group in comparison to Crude sericin powder solution- treated control group. The AST, ALT and ALP activities were significantly (P<0.001) decreased in omeprazole pretreated—aspirin- treated experimental groups when compared to aspirin- treated experimental groups. The results are shown in table no 2.

Effect of Sericulture product (SP) or Crude sericin powder solution on AST, ALT and ALP activity:
Table2:-

Group(n)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
Control group	50.83±0.08	56.42±0.07	59.23±0.09
Aspirin treated group	133.71±1.07***	132.22±0.12***	138.66±0.15***
SP+ Control Group	44.25±0.02*	46.47±0.03*	41.54±0.04*
SP+ Aspirin treated group	89.22±0.41**	91.92±0.06**	92.13±0.05**
Omeprazole + Control group	39.64±0.02*	41.77±0.04*	39.16±0.04*
Omeprazole + Aspirin treated group	84.11±0.11**	86.02±0.03**	83.33±0.05**

SP:Sericulture Product , ALP: Alkaline phosphatase, AST: Aspartate Aminotransferase,ALT: Alanine Aminotransferase, Values are mean±SEM, n=6, Data were analysed statistically using one way analysis of variance test followed by multiple comparison t-test,* P<0.001 when compared with control group,**P<0.001 when compared with Aspirin – treated group , ***P<0.001 when compared with other mentioned groups.

There was a sharp decline (P<0.001) in SOD activity both in stomach and duodenum tissues in the aspirin– treated experimental group as compared to the control group. The SOD activity was significantly (P<0.001) increased in Crude sericin powder solution- treated control group compared with the control group, both in stomach and duodenum tissues. Crude sericin powder solution significantly (P<0.001) increased SOD activity in Crude sericin powder solution- pretreated aspirin- treated experimental group in comparison to aspirin- treated experimental group, both in stomach and duodenum tissues. There was a sharp increase (P<0.001) in SOD activity both in stomach and duodenum tissues in the omeprazole- treated control group compared to the control group. The SOD activity was significantly (P<0.001) increased in the omeprazole- treated control group in comparison to Crude sericin powder solution - treated control group both in stomach and duodenum tissues. The SOD activity was significantly (P<0.001) increased in the omeprazole- pretreated aspirin- treated experimental group compared with the aspirin- treated experimental group both in stomach and duodenum tissues. The results are shown in table no 3.

Effect of Sericulture product (SP) or Crude sericin powder solution on Antioxidant enzymatic changes of stomach tissue:
Table 3:-

Group(n)	SOD(%inhibition unit)	LPO (nmol of [TBARS/-g] mol of tissue)	CAT (%inhibition unit)	GSH(µg/g of tissue)
Control group	13.66±0.07	5.11±0.01	14.75±0.01	32.79±0.03
Aspirin treated group	24.42±0.04***	11.99±0.02***	25.75±0.04***	3.44±0.02***
SP+ Control group	10.41±0.02*	2.37±0.02*	10.34±0.02*	37.46±0.06*
SP+ Aspirin treated Group	19.21±0.02**	8.65±0.03**	21.02±0.05**	11.34±0.05**
Omeprazole + Control group	15.57±0.02*	1.61±0.01*	8.92±0.02*	39.14±0.03*
Omeprazole + control group	17.33±0.02**	7.29±0.03**	19.02±0.03**	14.32±0.04**

SP:Sericulture Product , SOD:Superoxide Dismutase, LPO: Lipid peroxidation, CAT: Catalase, GSH: Glutathione, TBARS: Thio-barbituric acid reactive substances, Values are mean±SEM, n=6, Data were analysed statistically using one way analysis of variance test followed by multiple comparison t-test,* P<0.001 when compared with control group,**P<0.001 when compared with Aspirin – treated group , ***P<0.001 when compared with the other mentioned group.

There was a sharp rise (P<0.001) in LPO level both in stomach and duodenum tissues in the aspirin- treated experimental group as compared with the control group. The LPO level was significantly (P<0.001) decreased in Crude sericin powder solution- treated control group compared with the control group, both in stomach and duodenum tissues. Crude sericin powder solution significantly (P<0.001) decreased LPO level in Crude sericin powder solution- pretreated aspirin- treated experimental group in comparison to aspirin- treated experimental group, both in stomach and duodenum tissues. There was a sharp decline (P<0.001) in LPO level both in stomach and duodenum tissues in the omeprazole- treated control group when compared with the control group. The LPO level was significantly (P<0.001) decreased in the omeprazole- treated control group compared to the Crude sericin

powder solution- treated control group, both in stomach and duodenum tissues. The LPO level was significantly ($P<0.001$) decreased in the omeprazole- pretreated aspirin- treated experimental group when compared to the aspirin- treated experimental group, both in stomach and duodenum tissues.
The GSH level was significantly ($P<0.001$) increased in omeprazole- pretreated aspirin- treated experimental groups compared with aspirin- treated experimental groups both in stomach and duodenum tissues.

Discussion:-

The present study evaluates the protective role of Crude sericin powder solution on aspirin-induced gastric and duodenal ulcer in albino rat model with the possible involvement of the antioxidants. AST, ALT, and ALP are the most sensitive tests employed in the diagnosis of ulcer disease. Aspirin significantly increased UI, decreased MT, and decreased AST, ALT, and ALP activities in rat stomach and duodenal tissues, according to the results.The decrease of ALP activity seems to be a general property of all chemicals which are known to provoke severe ulcers. The activity of ALP may be relevant to duodenal ulcer pathogenesis or healing.The pathogenesis of ulcer disease is believed to reflect an imbalance between increased aggressive factors and decreased protective factors. The decreased ALP activity imbalances are one of the first lines of defence protecting the gastro-duodenal mucosa and the mucus bicarbonate barrier overlying the epithelium.The increase in UI decrease in MT, and decrease in AST, ALT, and ALP activities observed in the current study following oral administration of aspirin treatment could be attributed to gastro-protective failure. and repair mechanisms leading to disrupted mucosal barrier. It is evident from the results of the present investigation that treatment with Crude sericin powder solution significantly decreased the AST, ALT, and ALP activities at a dose of 400 mg/kg body weightChanges in LPO levels and antioxidant activities such as SOD, CAT, and GSH in stomach and duodenum tissues can explain these findings.The ability of gastric mucosa to resist injury by ingested irritants (aspirin) is attributed to a number of factors that have been collectively referred to as mucosal defence. The Crude sericin powder solution was found to have an excellent scavenging effect on LPO, which was well comparable with the standard drug omeprazole. So, from the results obtained on LPO levels, it may be concluded that the protection by Crude sericin powder may be due to vitamin E, beta-carotene, flavonols, and flavonoids, which are present in Crude sericin powder solution.

CAT is a defensive antioxidant enzyme that works in tandem with SOD. The harmful hydrogen peroxide is captured by CAT and converted into water and oxygen. Aspirin reduced CAT activity in rats. The inhibition of CAT activity during aspirin-induced ulcer may be due to an increase in reactive free radical production, which can cause oxidative stress in the cells.

The administration of organic Crude sericin powder solution inverted the CAT activity in liver tissues and protected from the free radical induced oxidative stress. This result supports that, the antioxidant properties of the organic drug was excellent as compared with the standard drug omeprazole. SOD, an important oxidative damage defence system, catalyses the destruction of superoxide radicals. According to our experimental results of the aforementioned antioxidant enzyme activities in stomach and duodenum tissues, aspirin significantly reduced SOD, CAT, and GSH activities in the aspirin-treated experimental group compared with control group, Crude sericin powder solution - treated control group, omeprazole- treated control group, omeprazole- pretreated aspirin- treated experimental group and Crude sericin powder solution - pretreated aspirin-treated experimental group. So, it may be concluded that the protection by Crude sericin powder may be due to some essential amino acids present in the Crude sericin powder solution.

In the present research work, we have observed the decreased level of glutathione in aspirin induced gastric and peptic ulcer rat models. Treatment with Crude sericin powder solution had significantly improved the level of reduced glutathione both in stomach and duodenum tissues. The standard drug omeprazole produced comparable results.. It prevents the hydroxyl radical generation by interacting with free radicals. The decreased level of GSH in the aspirin-treated experimental group seen in our study indicates that there was an increased generation of free radicals and the GSH was depleted during the process of combating oxidative stress. The essential amino acids present in Crude sericin powder solution are, capable of scavenging peroxyl radicals. Crude sericin powder is an oxidant and has powerful antiulcer activities. These compounds contain an OH group linked with the aromatic ring, and thus may possess potential antioxidant and antiulcer activities. The salient findings of our present study suggest that amino acids present in the Crude sericin powder solution provide a good source of antioxidants that could offer potential protective effects against aspirin- induced gastro–duodenal ulceration in albino rat model.

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