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Evaluation of Anti -Cataract Activity of Aqueous Extract of Shilajit Using In-Vitro Model on Goat Lens

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ABSTRACT

Cataract is defined as a clouding of the natural lens. Len is a part of the eye responsible for focusing and producing a clear sharp image. Cataract is caused due to degeneration & opacification of the lens fiber already formed. Shilajit is a natural mineral, a gift of nature's resource. Shilajit extract did not cause any mortality in mice up to the dose of 1 g/kg intraperitoneally. Goat eyeballs were used in the present study. They were obtained from the slaughterhouse. Glucose at a concentration of 55 mM was used to induce cataract, concentration of glucose (5.5 mM) served as normal control and Ascorbic Acid served as standard control. Incubation of lenses with glucose 55 mM showed opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the center, with complete opacification at the end of 72 hrs. Positive control (Ascorbic acid 40µg/ml) – lenses show slight degree of opacity, clear lens was not found. Test 1 (Shilajit20µg/ml) – lenses show slight degree of opacity, clear lens was not found. Test 2 (Shilajit20µg/ml) – lenses show slight degree of opacity, clear lens was not found. Test 3 (Shilajit40µg/ml) – Zero degrees opacity has occurred, clear lens is obtained. Test drug inhibits cataract genesis The Present investigation suggests that Shilajit treated groups have been shown to increase the content of water-soluble proteins, retarding the process of cataractogenesis initiated by high glucose concentration. Shilajit possesses anticataract activity due to the presence of antioxidant activities.

INTRODUCTION

Cataract is a major contributing factor to blindness. It is defined as a clouding of the natural lens, a part of the eye responsible for focusing and producing a clear sharp image It is called as a "peril of sight" because cataract has blinded more people throughout the ages than any other affliction of the eye. It is also called as "Senile cataract"[(1)].

Cataract is derived from the Latin word "cataracta" meaning waterfall. ARN (Age-Related Nuclear) Cataract is the most common form of cataract which is found in age more than 45 year and opacity forms in the center of the lens.

It is one of the leading cause of blindness worldwide, it accounts for approximately 42% of all blindness. More than 17 million people are blind because of cataract, and 28000 new cases are reported daily worldwide. Approximately 25% of the populations over 65 and about 50% over 80 have serious loss of vision because of cataract [(1)].

Cataract is associated with old age and cataract is a major complication of diabetes Mellitus because higher glycosylated hemoglobin levels are significantly associated with increased risk of cataract. Although many cataractogenic factors have been identified, the biochemical background of cataractogenesis is still unknown. It is a multifactorial disease occurs mainly due to the formation of large protein aggregates in the lens. The lens Na^+ - K^+ -ATPase activity plays an important role in maintaining lens transparency, and its impairment causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis[(2)].

There is a widespread belief that natural products are less toxic when they compared to pure chemicals. Recent data suggest that 80% of drug molecules are natural products or natural compound inspired [(3)].

According to the World Health Organization (WHO), traditional medicine (TM) incorporates health practices, approaches and knowledge of plant, mineral and animal-based medicines, applied singularly or in combination to treat and prevent illnesses or maintain well-being. WHO estimates that approximately 80 % of the earth's inhabitants depend on TM for their health needs. In this direction, enormous research is being conducted all over the globe with respect to plant-based medicines leaving the other two components of TM i.e., mineral and animal-based medicines [(4)]. Although majority of natural drugs are derived from plant and

animal origins, a few of them, obtained from mineral sources, like Shilajit, are of paramount significance as pharmaceutical Aids.

Shilajit is a natural mineral, a gift of nature's resource. *Shilajit*, is a herbo-mineral drug, contains ample amounts of fulvic acid and mineral constituents [(4)]. Shilajit is a pale-brown to blackish-brown exudation from rocks in Himalayan ranges of Indian subcontinent. It is also found in Nepal, Bhutan, Tibet, and China. Numerous traditional uses of Shilajit have been reported previously [(5)]. Shilajit is widely used in oriental medicine to arrest aging and to accelerate the process of rejuvenation the two major attributes of an Indian Ayurvedic and Siddha medicine.[(6)]

Reported pharmacology activities of shilajit are anti-inflammatory activity[7],analgesic activity[7], anti-diabetic activity[8] ,Immunomodulatory[9,10],Nootropic activity[11,12],anti-anxiety activity[11],anti-ulcer activity[13],anti-viral activity[9,10,14],Spermatogenic and ovogenic effects[15],antifungal activity[16], Protection of mast cells from degranulation[17],free radical scavenging and antioxidant effect[18] and anti-lipid-per oxidative activity[19].

PATENTS ON SHILAJIT:[20,21,22,23]

The Extensive research has been carried out on shilajit to justify its claims. In research study of Shilajit bioactive constituents proved that they have healing, anti-aging and restorative properties. The following is a list of patents so far filed on shilajit: US Patent No. 5,405,613 – vitamin/mineral composition. [(20)]US Patent application No. 20030198695 – Herbo-mineral composition [(21)]. US Patent No. 6,440,436 – Process for preparing purified shilajit composition from native Shilajit [(22)]; US Patent No. 6,558,712 – Delivery system for pharmaceutical, nutritional and cosmetic ingredients [(23)].

MATERIALS AND METHODS:

1. LITERATURE REVIEW:

An opacity of the lens or its capsule whether developmental or acquired is called a cataract. Cataract is caused due to degeneration & opacification of the lens fiber already formed .the formation of aberrant lens fiber or deposition of other material in their place [24].

World Health Organization (WHO) find out that around 285 million people in the world are visually impaired out of which 90.5 million of them are from the South-East Asian Region Of

the estimated 39 million blind people in the world, 90 percent are in developing countries; 22 percent in India alone. Therefore, the burden of blindness is largely in developing countries where 9 out of 10 of the world are blind live. Cataract alone is the cause of approximately 50 percent of the world's blindness.

A major complication of diabetes mellitus is cataract. Other risk factors associated are the loss of antioxidant mechanisms of lens, smoking, corticosteroids and many more. Though the biochemical background of cataractogenesis is still unknown, three biochemical factors are evident in the process of cataract formation;

1. First is hydration may be due to osmotic changes within the lens or due to changes in the semi-permeability of the capsule.

2. Second is denaturation of the lens protein with an increase in insoluble protein.

3. Third is slow sclerosis.

Normal lens contains sulfhydryl containing reduced glutathione and ascorbic acid as antioxidants. With the increase in age, this anti-oxidative mechanism becomes less effective and there is the increase in inactive insoluble proteins and semi-permeability of the lens capsule which may lead to cataract formation [25].

Cataractogenesis is influenced by multiple risk factors, like aging, diabetes mellitus, drugs, trauma, toxins, genetics, smoking and other ocular diseases. Multiple mechanisms for the development of cataract such as osmotic graduation, protein aggregates, oxidative stress, post-translational protein changes, phase separation are proposed for cataract formation. Combined factors of heritage, Ultra Violet light exposure, diet, some metabolic disorders, quality of life, cationic pump malfunction and lens metabolism disorder are believed to have a role in cataract formation. The increased incidences of cataracts, in diabetic patients, are also well.

2. SIGNS AND SYMPTOMS OF CATARACT:

As a cataract becomes more opaque, clear vision is compromised with loss of visual acuity. Contrast sensitivity is lost, so that shapes, shadows and color vision are less vivid. Veiling glare may occur when the light is scattered by the cataract into the eye. The affected eye will be a deficient red reflex. Contrast sensitivity test should be performed and if a loss in contrast

sensitivity is found then further consultation with an eye specialist would be recommended [26].

3. CAUSES OF CATARACT:

Various factors like day-light, diet, diabetes, dehydration, oxidation of lens proteins and peroxidation of lipids contribute to the generation of Len opacity in the older individuals. The various Nutritional deficiency, sunlight, smoking, environmental factors, lack of consumption of antioxidants can increase the risk of cataract. Still, it is known that Diabetes is a major cause of developing cataract [27].

4. CLINICAL SIGNIFICANCE OF SHILAJIT:

For nearly more than 3000 years Shilajit, a natural product, plays a vital role in soaring economic value in the folk medicine of the former Soviet Union and also in traditional Indian medicine and Tibetan pharmacology. It is also used as growth accelerator even for plants. In ancient Egypt, this resin was used for embalming mummies. Greek physicians used this medicine as an antidote to poisons and in the treatment of various problems including arthritis and inflammation. Currently, Shilajit is prohibited to be exported from the Soviet Union because it is being considered as a 'treasure of the country'.Among the numerous active principles of Shilajit, Fulvic acid and Humic substances are important. It is interesting to know about the beneficial use of Fulvic acid documented in the Chinese pharmacological compendium dating back to the 15th century. The compendium describes about a drug 'Wujinsan' containing Humic and Fulvic acids, implying that these substances are effective anti-inflammatory and blood-coagulating agents [28].

5. OBJECTIVE:

Today enormous research is being conducted all over the globe with respect to plant-based medicines leaving the other two components of Traditional Medicine i.e., mineral and animal-based medicines. Majority of natural drugs are derived from plant and animal origins, a few of them, obtained from mineral sources, like Shilajit, are of paramount significance as pharmaceutical aids.

An attempt has been made to evaluate the effect of Shilajit for Anticataract to find out the probable mechanism of action.

The present study is planned with the following objectives:

1) To elucidate the probable mechanism of Shilajit.

2) To compare the efficacy of Shilajit with the standard in dose-dependent manner.

3) To carry out Anticataract activity.

6. ACUTE TOXICITY OF SHILAJIT:[30]

Shilajit extract did not cause any mortality in mice up to the dose of 1 g/kg intraperitoneally. For the toxicological study, the experimental animals received the preparation daily in the form of 1–10 % aqueous solution (orally) for 1 month. The daily doses of Shilajit extract for rabbits and mice were 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, and 0.5 g/kg. On its application both once (0.5 g/kg) and on a multi-time basis (total dose was from 1.5 to 15 g/kg) the investigators did not observe any morphological or histological changes in the internal organs of animals in comparison with the control group. It was found that application of the remedy at the doses of 0.2 and 1 g/kg for 3 months did not lead to negative influence on the function of heart, liver, kidneys, blood cells, or nervous and endocrine systems.

The study of specific teratogenic action showed that treatment of pregnant rats with Shilajit did not render embryotoxic or teratogenic actions. The postnatal development of young rats, whose parents received the preparation, was also normal. Similarly, the effect of Shilajit on the development of mice embryo was studied. A total of 71 pregnant female mice were given Shilajit (250 and 500 mg kg -1) orally via needle tube, daily from day 8-12 of pregnancy. All the treated and control animals showed no differences in the number of the litter size, the placenta and the body weight of the embryos and the number of resorbed embryos at day 17 of gestation.

Few abnormalities were observed in both treated and control groups Nevertheless, the results of this study supports the safe use of Shilajit. Nearly every one of the investigators noted absence of side effects with Shilajit application at daily dose of 0.1–0.3 g inwardly. A few subjects with bone fractures felt burning sensation in the region of fracture. Few felt a sense of heat and subjects with chronic colitis, reported burning, weakness, and sweating for 40–60 min. after application of Shilajit extract. At higher doses (0.9-1.5 g/d) it can lead to increase in body temperature to 37.5^0C, sweating, and headache. The duration of this reaction was

from 20 min. to 2–3 hours. The concentrations of lead, mercury, and arsenic (µg/g) in Shilajit formulation manufactured by Syncom Company, India available in Boston area, USA was measured by x-ray fluorescence spectroscopy. The analysis reveals the presence of 8 µg/g of lead which is of great concern because the permissible level of lead is less than 5 µg/g [(28)].

7. PREPARATION AND SCHEDULED:

The selected doses of Shilajit 20, 40, 80µg/ml for the goat lens and Ascorbic Acid 40 µg/ml was used as standard drug dose.

8. EXPERIMENTAL PROCEDURE:[31]

- **COLLECTION OF EYEBALLS:**

Goat eyeballs were used in the present study. They were obtained from the slaughterhouse. Immediately after slaughter and transported to laboratory at 0-4 degree Celsius [(30)].

- **LENS CULTURE:**

Fresh goat eyeballs were obtained from the slaughterhouse and immediately transported to the laboratory at 0-4°C. The lens were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, $NaHPO_4$ 0.5 mM, $CaCl_2$ 0.4 mM and glucose 5.5 mM) at room temperature and maintain pH 7.8 by addition of $NaHCO_3$). Penicillin G 32% and streptomycin 250 mg% added to the culture media to prevent bacterial contamination. At high concentration, glucose in the lens was metabolized through sorbitol pathway and accumulation of polyol causing overhydration and oxidative stress. This lead to cataractogenesis.

- **INDUCTION OF IN VITRO CATARACT:**

Glucose at a concentration of 55 mM was used to induce cataracts. At high concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyol (sugar alcohols) causes overhydration and oxidative stress. This generates cataractogenesis. These lenses were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM) served as normal control and 55 mM served as toxic control) for 72 hours.

- **STUDY DESIGN AND GROUPS:**

Goat lenses were divided into six groups of six lenses each and incubated as following table no 1:

Table No. 1: Treatment groups

Group No	Group Name	Treatment	Drug Dose
I.	Normal Control	Aq. Humor + Glucose 5.5 mM	-
II.	Negative Control	Aq. Humor + Glucose 55 mM	-
III.	Standard	Aq. Humor + Glucose 55 mM + Ascorbic Acid	40 µg/ml
IV.	Test 1	Aq. Humor + Glucose 55 mM + Shilajit	20µg/ml
V.	Test 2	Aq. Humor + Glucose 55 mM + Shilajit	40 µg/ml
VI.	Test3	Aq. Humor + Glucose 55 mM +Shilajit	80 µg/ml

- **PHOTOGRAPHIC EVALUATION:**

Lenses were placed on a wired mesh with the posterior surface touching the mesh, the pattern of mesh number of squares clearly visible through the lens was observed to measure lens opacity. The degree of opacity was graded as follows:

‘0’- absence of opacity.

‘1’- slight degree of opacity.

‘2’’-presence of diffuse opacity.

‘3’’-presence of extensive thick opacity

- **PREPARATION OF LENS HOMOGENATE:**

After 72 hours of incubation, homogenate of lens was prepared in tris buffer (0.23 M, pH-7.8) containing 0.25 × 10-3 M EDTA and homogenate was adjusted to 10% w/v which was centrifuged at 10,000 G at 4ºC for 1hour and the supernatant was used for the estimation of biochemical parameters.

9. BIOCHEMICAL PARAMETER: [31]

- **ESTIMATION OF TOTAL PROTEIN CONTENT :**

To 0.1 ml of lens homogenate, 4.0ml of alkaline copper solution was added and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as μg/mg lens tissue.

10. STATISTICAL ANALYSIS:

All data were expressed as mean ± SD. All data were analyzed with Prism Pad software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Dennett's test. The values are expressed as mean ± S.D. and results were considered significantly different if $P<0.05$.Statistical variations are compared as follows: Normal Goat lens vs. Goat lens + Glucose 55mM, Goat lens + Glucose55mM vs. Goat lens + Glucose55mM +Shilajit.

RESULTS AND DISCUSSION

- **IN- VITRO ANTICATARACT ACTIVITY:**

Incubation of lenses with glucose 55 mM showed opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the center, with complete opacification at the end of 72 hrs.

- **PHOTOGRAPHIC EVALUATION:**

After 72 hours of incubation, transparency was maintained in the Group I (normal control group) [fig.A] but there was the complete loss of transparency in the Group II (negative control group) [fig.B] indicating complete cataractogenesis. Group III (positive control group) [fig.C] containing lens treated with standard ascorbic acid were squares of the graph paper were visible through the lenses. Goat lenses of groups containing escalated doses of the Shilajit (Group IV, V, and VI) were less hazy and the squares of the graph paper were visible through the lenses indicating suppression of cataract formation [(D, E, and F)]. Group VI

(containing 80µg/ml) was more effective in suppressing cataract formation [fig (F)] than Group IV [fig.(D)] and Group V [fig.(E)].

Fig (A): Normal control (group I)

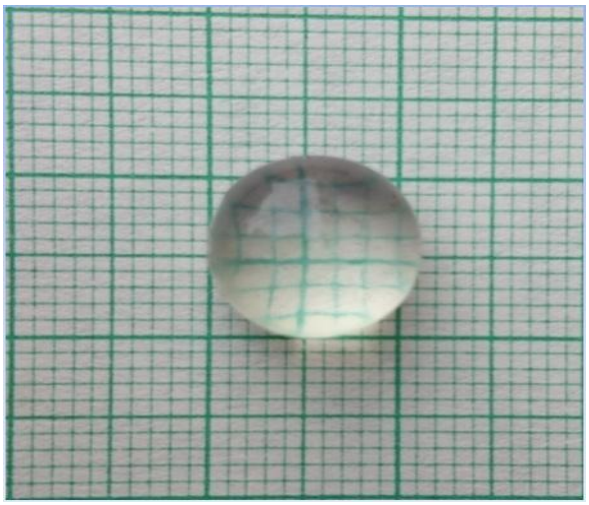

Fig (B): Negative control (Group II) showed Showed complete cataractogenesis transparent lens.

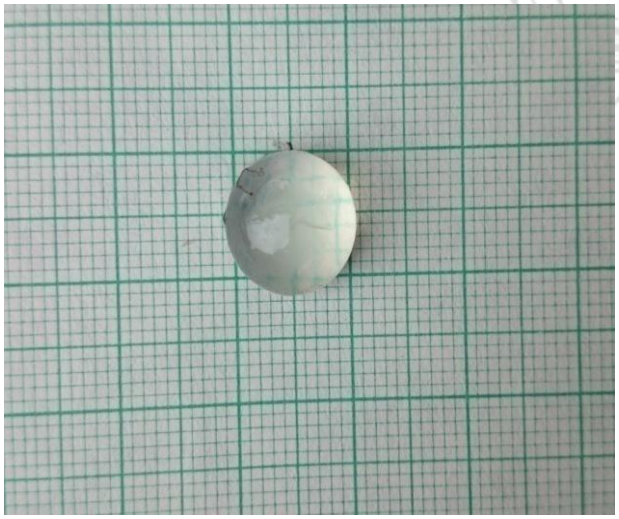

Fig (C): Positive control (Group III)

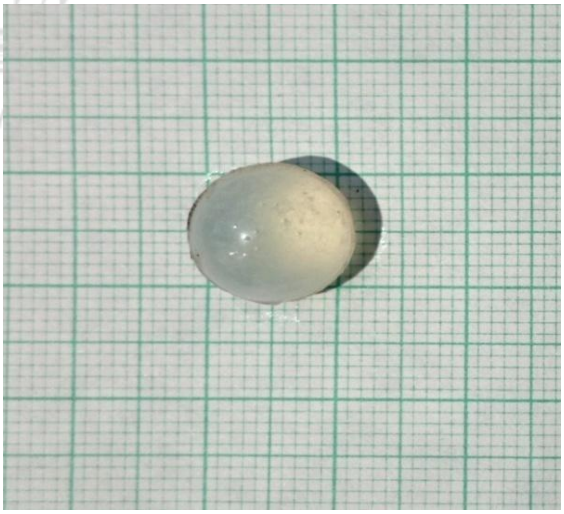

Fig (D): Group IV

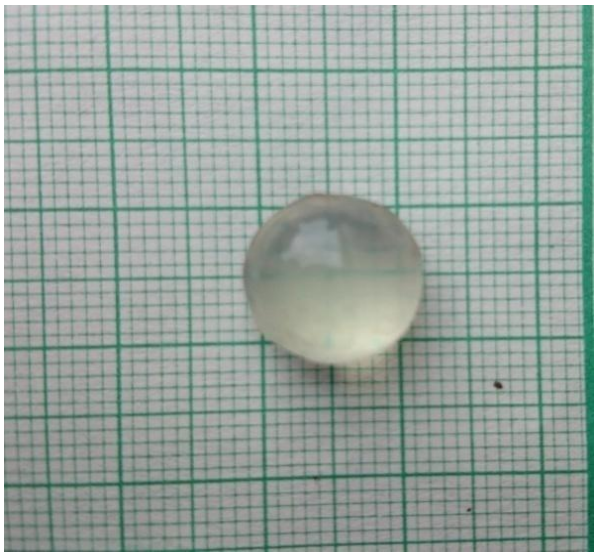

Fig (E): Group V

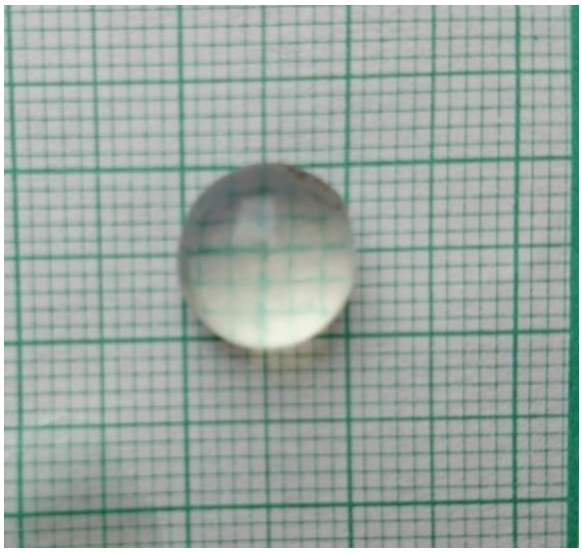

Fig. (F): Group VI

In normal control, the lens was incubated for 72 hr. in aqueous humor and 5.5 mM of glucose conc. The clear lens because of low conc. of glucose does not show any effect on lens and numbers of squares are clearly visible through the lens.

After 72 hr. of incubation of lens in aqueous humor and 55 mM of glucose, the lens induces high conc. of glucose in the lens which metabolized through sorbitol pathway and accumulation of polyol causing hydration and oxidative stress. This leads to cataractogenesis. The lens showed extensive thick opacity.

After 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose + 40µg/ml Ascorbic acid std. drug, numbers of squares were not clearly visible through lens as compared to test drug VI, the lens shows the slight degree of opacity.

After 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose +20 µg/ml and 40µg/ml Shilajit test drug, number of squares were not clearly visible through the lens as compared to Shilajit 80 µg/ml test drug, the lens show a slight degree of opacity.

After 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose + 80 µg/ml Shilajit test drug, number of squares were clearly visible through the lens. The lens showed the absence of opacity because the test drug inhibits cataractogenesis and oxidative stress.

In the in-vitro model for inducing cataract using glucose concentration 55 mM provide an effective model on isolated lenses of goat. Incubation of goat lenses in the media containing

high glucose (55 mM) concentration has induce cataract and has shown to cause the considerable drop in Na^+/ K^+- ATPase activity, with the progression of opacity. The impairment of Na^+/K^+- ATPase causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis. This alteration in the Na^+, K^+ ratio changes the protein content of the lens, leading to a decrease in total proteins causing lens opacification. The imbalance of Na^+ and K^+ was prevented due to an action of Shilajit which corrects imbalances in the polyol pathway by decreasing aldose reductase activity, sorbitol concentration, and intracellular glucose.

Table No. 2: Effect of Shilajit on degree of opacity on lens by glucose-induced cataract:

Sr. No.	Compound	Degree of opacity
1	Normal	0
2	Negative control (Glucose 55 mM)	3
3	Positive control (Ascorbic acid 40µg/ml)	1
4	Test 1(Shilajit20µg/ml)	2
5	Test 2 (Shilajit 40µg/ml)	1
6	Test 3 (Shilajit80µg/ml)	0

Normal control - Zero degree opacity occurred, clear lens is obtained.

Negative control – the presence of extensive thick opacity, because of high conc. of glucose-induced cataractogenesis.

Positive control (Ascorbic acid 40µg/ml) – lenses show the slight degree of opacity, clear lens was not found.

Test 1 (Shilajit20µg/ml) – lenses show the slight degree of opacity, clear lens was not found.

Test 2 (Shilajit20µg/ml) – lenses show the slight degree of opacity, clear lens was not found.

Test 3 (Shilajit40µg/ml) – Zero degree opacity is occurred, the clear lens is obtained. Test drug inhibits cataractogenesis.

Shilajit was evaluated against glucose-induced cataract on goat eye. Shilajit significantly protected the lens morphology and activity and clarity: 50% of the eyes had almost clear lenses; in contrast, 100% of the negative control eyes developed dense nuclear opacity. From the current study, it is evident that Shilajit protects the lens against oxidative stress. These

results in glucose-induced cataracts in vitro studies not only demonstrate the protective effect of Shilajit but also indicate that it prevents cataractogenesis by virtue of its antioxidant properties. Shilajit, therefore, may be useful for prophylaxis or therapy against cataract. After 72 hr. of incubation in glucose 55 mM, the lens becomes completely opaque as against lenses in normal control. Incubation of lenses with Shilajit and ascorbic acid both the concentrations were used, which seem to retard the progression of opacification compared with lenses incubated in glucose 55 mM (Negative Control). The effect of Shilajit, on the positive control groups, showed considerable retardation in the progression of lens opacification which is near normal when compared to negative control.

Table No. 3: Effect of Shilajit on Protein levels (total proteins and water-soluble proteins) in Goat lens homogenate after 72 hours of incubation in glucose 55 mM induced cataract:

Group No.	Treatment	Total proteins [mg/gm]	Water-soluble proteins [mg/gm]
1	Normal lens Control (Glucose 5.5 mM)]	220.51 ± 2.239	80.656± 3.040
2	Glucose 55 Mm	170.40±2.095$^{\#\#}$	61.249± 3.054$^{\#\#}$
3	Glucose 55 mM + Ascorbic acid 40µg/ml	210.37± 2.649**	76.514± 2.148**
4	Glucose 55 mM + Shilajit20µg/ml	182.24± 2.139	70.889± 1.279*
5	Glucose 55 mM + Shilajit40µg/ml	208.31± 1.648**	74.821± 1.643**
6	Glucose 55 mM + Shilajit80µg/ml	213.76± 1.687**	77.648± 1.149**

N=6, values are expressed as Mean ± SEM. Comparison were made as follows, # $p < 0.05$, ## $p < 0.01$ when compared with normal control. * $p < 0.05$, ** $p < 0.01$ when compared with negative control. (Values are compared on 72hr by one way ANOVA Dennett test) N.S. – nonsignificant.

Glucose 55 mM treated lenses (Group-II) showed significantly low concentrations of proteins (total and water-soluble proteins) in the lens homogenate ($P<0.01$) compared with normal lenses (Group-I). Ascorbic acid treated lenses (Group-III) and Lenses treated with Shilajit (Group-IV, V, VI) showed higher concentrations of proteins (total and water-soluble proteins) ($P<0.01$) compared with Glucose 55 mM treated lenses (Group-II).

CONCLUSION

The Present investigation suggests that Shilajit treated groups have been shown to increase the content of water-soluble proteins, retarding the process of cataractogenesis initiated by high glucose concentration. Shilajit possesses Anticataract activity due to the presence of antioxidant activities which might be helpful in preventing and/or slowing cataract formation.

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REFERENCES

1)Shah NK, Patel PK, Vyas BA, Joshi SV. "Evaluation of Anti-Cataract Activity of *Asparagus Racemoss* Root Extract Using In-Vitro Model of Goat Lens". *International Journal for Pharmaceutical Research Scholars.* (2013); 2(3): 19-26.
2)Manish Kumar, Talever Singh, Javed Ali and L.K Tyagi. "In Vitro Anticataract Activity of *Zingiber officinale* on Goat Lenses".*International Journal of Pharmaceutical & Biological Archives*. (2011); 2(5):1430-1433.
3)Sahid Aziz, MahanjitKonwar, Swarnamoni Das. "In-Vitro Anticataract Activity of *Hibiscus rosa-sinensis* Linn on Goat Lens".*Int J Pharm Pharm Sci*. (2015); 7(5): 334-336.
4)Wilson E, Rajamanickam GV, Dubey GP, Klose P, Musial F, Saha FJ, Rampp T, Michalsen A, Dobos GJ. "Review on *Shilajit* used in traditional Indian medicine". *J Ethnopharmacol.* (2011);136(1):1-9.
5)HarsahayMeena, H. K. Pandey, M. C. Arya, and Zakwan Ahmed. "*Shilajit:* A panacea for high-altitude problems".*Int J Ayurveda Res*. (2010); 1(1): 37-40.
6)AnuyaRege, ParikshitJuvekar, ArchanaJuvekar. "In Vitro Antioxidant and Anti-Arthritic Activities of *Shilajit*".*Int J Pharm Pharm Sci*. (2012); 4(2): 650-653
7)Acharya SB, Frotan MH, Goel RK, Tripathi SK, Das PK. "Pharmacological actions of Shilajit". *Indian J Exp Biol*. (1988); 26: 775–777.
8)Bhattacharya SK. "*Shilajit* attenuates streptozotocin-induced diabetes mellitus and the decrease in pancreatic islet superoxide dismutase activity in rats".*Phytotherapy Res.*(1995); 9(1): 41-44.
9)Ghosal S. "Chemistry of *Shilajit*, an immunomodulatory Ayurvedic rasayan". *Pure ApplChem (IUPAC)* (1990); 62: 1285–1288.
10)Bhaumik S, Chattapadhay S, GhosalS. "Effects of *Shilajit* on mouse peritoneal macrophages". *Phytother Res*. (1993); 7: 425–427.
11)Jaiswal AK, Bhattacharya SK. "Effects of *Shilajit* on memory, anxiety and brain monoamines in rats". *Indian Journal of Pharmacology.* (1992); 24: 12-17.
12)Ghosal S, Lal J, Jaiswal AK, Bhattacharya SK. "Effects of *Shilajit* and its active constituents on learning and memory in rats". *Phytother Res.* (1993); 7: 29–34.
13)Goel RK, Banerjee RS, Acharya SB. "Antiulcerogenic and antiinflammatory studies with Shilajit".*Journal of Ethnopharmacology*. (1990); 29:95-103.
14)Ghosal S. "Free radical oxidative stress and antioxidative defense". *Phytomedica. (*2000); 21(1&2): 1-8.
15)Jeong- Sook Park, Gee-Young Kim, Kun Han, "The spermatogenic and ovogenic effects of chronically administered *Shilajit* to rats", *Journal of Ethnopharmacology.* (2006); 107: 349-353.

16)Shaliniet. "Al. Antifungal activity screening and HPLC analysis of crude extract from Tectonagrandis, *Shilajit*, Valerianawallachi".*Electrical Journal of Environment, Agricultural and Food Chemistry.* (2009); 8(4): 218-229.
17)Ghosal S, Lal J, Singh SK, Dasgupta G, Bhaduri J, Mukopadhyay M, Bhattacharya SK. "Mast cell protecting effects of *Shilajit* and its constituents".*Phytotherapy Res*. (1989); 3(6): 249-252.
18)Ghosal S, Lata S, Kumar Y, Gaur B, Misra N. "Interaction of *Shilajit* with biogenic free radicals". *Indian J Chem.*(1995); 34b: 596–602.
19)Tripathi YB, Shukla S, Chaturvedi S."Anti-lipid peroxidative property of *Shilajit*". *Phytotherapy Res*. (1996); 10(3): 269-273.
20)Rowland D. *Vitamin/mineral composition*. US Patent No. 5,405,613.(1995)
21)Ghosal S. *Herbo-Mineral compositions*. US Patent application No. 20030198695. (2002a)
22)Ghosal S. Process for preparing purified shilajit from native shilajit. US Patent No. 6,440,436.(2002b)
23)Ghosal S.Delivery system for pharmaceutical, nutritional and cosmetic ingredient. US Patent No. 6,558,712.2003.
24)Sahid Aziz, MahanjitKonwar, Swarnamoni Das. "In-Vitro Anticataract Activity of *Hibiscus rosa-sinensis* Linn on Goat Lens". *Int J Pharm PharmSci.* 7(5): 334-336.
25)Shah NK, Patel PK, Vyas BA, Joshi SV. " Evaluation of Anti-Cataract Activity of *Asparagus racemosus* Root Extract Using In-Vitro Model of Goat Lens". *International Journal for Pharmaceutical Research Scholars.* (2013);2(3): 19-26.
26)Allen D and Vasavada A. "Cataract and surgery for cataract". *BMJ*.(2006); 333(7559): 128-132.
27)Kothadia AD, Shenoy AM, Shabaraya AR, Rajan MS, Viradia UM, Patel NH. "Evaluation of cataract preventive action of phycocyanin". *Int J Pharm Sci Drug Res.* (2011); 3(1): 42-44.
28)Wilson E, Rajamanickam GV, Dubey GP, Klose P, Musial F, Saha FJ, Rampp T, Michalsen A, DobosGJ."Review on shilajit used in traditional Indian medicine." *J Ethnopharmacol*. (2011) 14;136(1):1-9.
29)Mittal P., Kaushik D., Gupta V., Bansal P., Khokra S. " Therapeutic Potentials of "*Shilajit Rasayana"*-A Review". *IJPCR, (*2009),1(2) (47-49).
30)Pravin Ramdas Adsul, prof. Hemant Jibhau Pagar and dr. Pandurang Madhukarrao Gaikwad: a study on preliminary photochemical, acute toxicity and diuretic potential of leaves of *Mangifera indica*. World journal of pharmacy and pharmaceutical sciences2017; 6: 1424-1432.
31) Manish Kumar, Talever Singh, Javed Ali and L.K Tyagi. "In Vitro Anticataract Activity of *Zingiber officinale* on Goat Lenses".*International Journal of Pharmaceutical & Biological Archives*(2011); 2(5):1430-1433.