EVALUATION OF GLUCOSE LOWERING, HEPATOPROTECTIVE AND HYPOLIPIDEMIC ACTIVITIES OF ETHANOLIC EXTRACT OF SEEDS OF ERIobotryA japonIca IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Sabeeha Shafi* and Nahida Tabassum

Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, Kashmir, Jammu & Kashmir (India).

ABSTRACT

Background: Diabetes is India’s fastest growing disease nowadays. About 72 million cases are recorded in 2017 and the figure is expected to nearly double by 2025. There is urgent need to find a herbal drug which can lower the complications related to diabetes. Aim: The present study was undertaken to screen the glucose lowering, hepatoprotective and hypolipidemic activities of ethanolic extract of seeds of Eriobotrya japonica in streptozotocin induced diabetic rats. Methods: First of all, the phytochemical screening of the extract was done. The animals were divided into five groups. The first group was Normal Control group which received only the vehicle. The 2nd group was toxic group which included those animals in which diabetes was induced by streptozotocin. The 3rd group were those animals which received streptozotocin and standard antidiabetic drug-glibenclamide. 4th group was diabetic animals receiving 50 mg/kg b.w dose of seeds of Eriobotrya japonica. 5th group included those diabetic animals which received 100mg/kg b.w of the plant extract. The biochemical parameters that were evaluated were blood glucose levels, liver function tests and lipid profile tests. At the end, the animals were sacrificed and histopathology of pancreas and liver was also done. Results: The ethanolic extract showed presence of various phytochemicals. The results showed significant decrease in blood glucose levels, lipid profile and liver function tests in animals treated with different doses of the plant extracts. Conclusion: The phytochemical screening revealed the presence of various bioactive components such as...
alkaloids, glycosides and flavonoids. It can be assumed that the potential pharmacological activity might be due to the presence of phytochemicals present in the seeds.

**KEYWORDS:** *Eriobotrya japonica* seeds, glucose lowering, hepatoprotective, hypolipidemic.

**INTRODUCTION**

Diabetes mellitus is taking the shape of an epidemic in India. As the salaries have increased and all the socio-economic people have experienced a rise in living standards, diabetes, a metabolic disease with hyperglycaemia leads to many complications like diabetic neuropathy, diabetic nephropathy, diabetic retinopathy and many other complications. WHO defines the disease as the 7th cause of death in 2030. India currently represents 49% of the world’s diabetes burden, with an estimated 72 million cases in 2017, this figure is expected to almost double to 134 million by 2025. This disease can lead to cardiovascular disease which doubles the risk of death.[1-2]

Searches are going on to understand and manage diabetes mellitus because the disease related complications are increasing day by day. There is presence of large number of medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat and this disease. Literature shows India has 45,000 plant species and several thousand have medicinal properties. Data shows more than 800 plant species have anti-diabetic activity. There has been great demand for herbal products due to easy availability, lesser side effects and low cost. For this purpose, herbal plants are scrutinized continuously and explored for their effect as antidiabetic agents.[3-10]

One of the plants is *Eriobotrya japonica* locally known as loquat, has been used since olden times in the ethno medicine for treating diseases. Although it is native to China and Japan, it grows in many parts of the world including India. One variety is found in Kashmir also. It is an evergreen tree which is having many medicinal uses. The leaves are of great importance and have been used to treat nausea, vomiting, belching, hiccups and gastro-intestinal disorders. The flowering period of this plant is from April to June. The reported bioactive compounds of this fruit include flavonoids, triterpenic acids, carotenoids, volatile compounds which attribute to aroma, oleanolic acid and ursolic acid. The reported pharmacological activities include anti-inflammatory anti-oxidant, anti-mutagenic, anti-viral, and other activities. The present study was aimed to investigate glucose lowering, hepatoprotective and
hypolipidemic activity of ethanolic extract of *Eriobotrya japonica* seeds in streptozotocin induced diabetic rats.[11-21]

**MATERIALS AND METHODS**

**Plant Material**
The seeds were separated from the fruits of *Eriobotrya japonica* (family Rosaceae) which were collected from Shalimar area of the Srinagar district. The period of collection was during the months of April to June. The aerial part containing the leaves and fruits were authenticated by a plant taxonomist in the Centre of Plant Taxonomy, University of Kashmir, Srinagar. This identification was done on the basis of the features as described by Kirtikar and Basu in 1935. A sample of this plant having fruit and leaves was deposited in the herbarium of the Department of Taxonomy, University of Kashmir under voucher specimen number 1012(KASH) dated 15-09-2008 for future reference. The fruits were dried in a well-ventilated room. The outside temperature ranged between 18 to 32°C.

**Preparation of the extract**
After drying, the seeds were separated from the fruits. They were coarsely powdered. 500 gm of the seeds were allowed to macerate for 48 hrs with 50% ethanol, with occasional shaking. The ethanolic extract was filtered through Whatmans filter paper after 48 hours. The extract was then macerated again with fresh 50% ethanol. The filtrate obtained from the first and the second maceration was then combined and the solvent used was recovered. The extract was then evaporated to dryness after the recovery of the alcohol. The yield was noted. The extract was refrigerated at 4°C for future use in experimental studies.

**Phytochemical Screening**[22-24]
The ethanolic extract obtained was subjected to qualitative tests for identification of different constituents like tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins and steroids. This was done by using simple and standard qualitative methods described by Trease and Evans.

**Pharmacological Study**[25]

**Animals**
Albino healthy rats of either sex weighing about 180-210 g were used. These animals were procured from Central Animal House, IIIM (Indian Institute of Integrative Medicine) Jammu. They were housed in clean polypropylene cages. The rats were acclimatized for a period of 7
days before starting the experiment. Standard environmental conditions such as temperature ranging from 18 to 32° C, relative humidity (70%) and 12 hrs dark/light cycle were maintained in the quarantine. All these animals were fed with rodent pellet diet (Ashirwad Industries) and were given water *ad-libitum* under strict hygienic conditions. All procedures were performed in accordance to CPCSEA guidelines after approval from the Institutional Animal and Ethics Committee (IAEC) of the Department of Pharmaceutical Sciences, University of Kashmir[No. F-IAEC (Pharm.Sc) APPROVAL.]

**Induction of Diabetes**

Diabetes was induced by administering a single dose of streptozotocin (STZ) 50mg/kg b.w., which was freshly dissolved in 0.1 M citrate buffer (pH.4.5) and injected intraperitoneally within 15 minutes of dissolution in a vehicle volume of 0.4 ml with 1 ml of tuberculin syringe fitted with 24 gauge needle. Diabetes was confirmed on 3*rd* day post administration of streptozotocin by estimating the fasting blood glucose concentration. During this period these animals are given free access to water. Fasting blood glucose level is checked by glucostrips. The rats having blood glucose levels > 250 mg/dl were separated and selected for further studies. The animals are given the following treatment.

Group I. Normal Control receiving 2% of gum acacia.
Group II. Diabetic Control which received STZ 50mg/kg b.w single dose i.p
Group III. STZ + Glibenclamide (3 mg/kg)
Group IV. STZ+ EBJS [50mg/kg.b.w]
Group V. STZ+ EBJS [100mg/kg.b.w]

The experiment was started on the same day except normal control and diabetic control rats for a period of 15 days orally. These animals were given free access to standard diet and water during this period. Fasting blood glucose levels were estimated on 1*st*, 4*th*, 9*th* and 15*th* day of the treatment. On the 16*th* day, blood samples were collected from overnight fasting animals by cardiac puncture. The rats were anaesthesized by mild ether anaesthesia before cardiac puncture. The blood sample which was collected was kept aside for 30 minutes for clotting. By centrifuging the sample at 6000 r.p.m for 20 minutes, the serum was separated and analyzed for various biochemical parameters.
Statistical Analysis
The data obtained from the biochemical estimations is expressed as Mean ± SEM for each group. After this the statistical analysis was carried out using one way analysis of variance (ANOVA) followed by student t test. Values p> 0.05 were considered non significant, p< 0.05 as significant, p< 0.01 as highly significant and p<0.001 as very highly significant respectively.

The biochemical parameters were estimated as per the following methods.

**Biochemical parameters evaluated were**\[^{[26-32]}\]

a) Serum Glucose Levels

b) Lipid Profile
   i. Serum Total Cholesterol Levels
   ii. Serum Triglycerides Levels
   iii. Serum HDL Cholesterol Levels
   iv. Serum LDL Cholesterol Levels

c) Liver Function Tests
   i. Serum Bilirubin Levels
   ii. SGOT levels
   iii. SGPT levels
   iv. Serum Total Proteins
   v. Serum Albumin
   vi. Serum Alkaline Phosphatase

d) Body Weight

e) Histopathology\[^{[33-34]}\]

At the end of the experiment, the animals were sacrificed and pancreas and liver was taken out. Histopathology of the pancreas and liver was also done.

The organs were taken out, preserved in 10% formalin and sent for histopathological studies.

**RESULTS**

**Percentage yield**

**Ethanolic extract of Eriobotrya japonica seeds**
Weight of the dried seeds taken = 1000 gms
Weight of the extract obtained = 110 gms
% yield = \(\frac{\text{Weight of the extract obtained}}{\text{Weight of the dried Seeds taken}} \times 100\)

% age yield of the ethanolic extract = 11%

<table>
<thead>
<tr>
<th>Extract</th>
<th>Colour</th>
<th>Odour</th>
<th>% Extractive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Ethanolic</td>
<td>Dark Brown</td>
<td>Characteristic</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 1: Effect of different doses of ethanolic extract of *Eriobotrya japonica* seeds (EBJS) on Serum Glucose Levels (mg/dl) and lipid profile against Streptozotocin (STZ) induced diabetes mellitus in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Serum Glucose Levels (mg/dl)</th>
<th>Serum Cholesterol Levels (mg/dl)</th>
<th>Serum Triglycerides Levels (mg/dl)</th>
<th>HDL Cholesterol Levels (mg/dl)</th>
<th>LDL Cholesterol Levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Control (0.2 ml of 2% gum acacia)</td>
<td>77.62±4.96</td>
<td>88.22±2.01</td>
<td>76.71±3.45</td>
<td>33.07±2.15</td>
<td>42.51±2.35</td>
</tr>
<tr>
<td>Group II</td>
<td>Diabetic control (STZ)</td>
<td>207.5±2.97</td>
<td>195.18±3.54</td>
<td>193.01±4.84</td>
<td>18.88±2.62</td>
<td>87.02±3.07</td>
</tr>
<tr>
<td>Group III</td>
<td>STZ + Std Antidiabetic drug Glibenclamide (3mg/kg)</td>
<td>129.56±12.97</td>
<td>193.34±5.69</td>
<td>186.96±4.31</td>
<td>19.75±1.95</td>
<td>86.26±3.02</td>
</tr>
<tr>
<td>Group IV</td>
<td>STZ+ EBJS (50mg/kg)</td>
<td>181.75±4.03</td>
<td>182.54±1.97</td>
<td>152.34±3.6</td>
<td>26.11±3.73</td>
<td>72.26±2.67</td>
</tr>
<tr>
<td>Group V</td>
<td>STZ+ EBJS (100mg/kg)</td>
<td>150.22±5.34</td>
<td>130.35±3.3</td>
<td>134.69±3.3</td>
<td>26.48±1.86</td>
<td>63.21±3.62</td>
</tr>
</tbody>
</table>

STZ Dissolved in 0.1M citrate buffer at a. dose of 50mg/kg b.w and injected i.p. single dose
Diabetes confirmed on third day post administration of streptozotocin. Standard drug
Glibenclamide & plant given as ethanolic extracts were administered orally for 15 days, in a single dose daily after confirmation of hyperglycaemia.

n = 6 (Number of animals in each group)

Group II is compared with Group I and all other groups are compared with group II

*** p < 0.001 Very highly significant; ** p< 0.01; Highly significant;
Table 2: Effect of different doses of ethanolic extract of *Eriobotrya japonica* seeds (EBJS) on liver function tests against Streptozotocin (STZ) induced diabetes mellitus in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Serum Bilirubin Levels (mg/dl)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>Serum Total Proteins Levels (gm/dl)</th>
<th>Serum Albumin Levels (gm/dl)</th>
<th>Serum Alkaline Phosphatase Levels (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Control (0.2 ml of 2% gum acacia)</td>
<td>0.55±0.05</td>
<td>21.61±1.31</td>
<td>26.06±1.78</td>
<td>7.13±0.31</td>
<td>2.65±0.09</td>
<td>78.20±4.6</td>
</tr>
<tr>
<td>Group II</td>
<td>Diabetic control (STZ)</td>
<td>3.11±0.23</td>
<td>32.12±4.23</td>
<td>40.72±2.28</td>
<td>4.79±0.33</td>
<td>1.16±0.19</td>
<td>92.88±2.58</td>
</tr>
<tr>
<td>Group III</td>
<td>STZ + Std Antidiabetic drug Glibenclamide (3mg/kg)</td>
<td>2.54±0.15</td>
<td>31.77±1.46</td>
<td>38.13±1.97</td>
<td>5.41±0.26</td>
<td>1.57±0.13</td>
<td>86.92±1.59</td>
</tr>
<tr>
<td>Group IV</td>
<td>STZ+ EBJS (50mg/kg)</td>
<td>2.83±0.11</td>
<td>28.39±1.26</td>
<td>28.13±1.02</td>
<td>4.92±0.18</td>
<td>1.46±0.18</td>
<td>91.31±3.84</td>
</tr>
<tr>
<td>Group V</td>
<td>STZ+ EBJS (100mg/kg)</td>
<td>1.55±0.1</td>
<td>21.9±1.51</td>
<td>23.45±0.99</td>
<td>5.93±0.31</td>
<td>1.68±0.03</td>
<td>87.87±5.04</td>
</tr>
</tbody>
</table>

STZ Dissolved in 0.1M citrate buffer at a. dose of 50mg/kg b.w and injected i.p. single dose
Diabetes confirmed on third day post administration of streptozotocin. Standard drug Glibenclamide & plant given as ethanolic extracts were administered orally for 15 days, in a single dose daily after confirmation of hyperglycaemia.

n = 6 (Number of animals in each group)
Group II is compared with Group I and all other groups are compared with group II

*** p < 0.001 Very highly significant; ** p< 0.01; Highly significant;

Table 3: Effect of ethanolic extract of *Eriobotrya japonica* seeds(EBJS) on Average Body Weight (gms) against Streptozotocin induced diabetes mellitus in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Average Body weight (in gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DAY 1</td>
</tr>
<tr>
<td>I</td>
<td>Normal control 0.2 ml of 2% gum acacia</td>
<td>250.58±8.14</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control 0.2 ml of 2% gum acacia</td>
<td>205.83±7.64</td>
</tr>
<tr>
<td>III</td>
<td>STZ + Std drug Glibenclamide (3mg/kg. b.w)</td>
<td>203.17±4.91</td>
</tr>
<tr>
<td>VIII</td>
<td>STZ + EBJS (50 mg/kg b.w)</td>
<td>248.98±3.06</td>
</tr>
<tr>
<td>IX</td>
<td>STZ + EBJS (100 mg/kg b.w)</td>
<td>213.73±7.14</td>
</tr>
</tbody>
</table>
HISTOPATHOLOGY RESULTS

Effect of Ethanolic extract of *Eriobotrya japonica* seeds on histopathology of different organs against streptozotocin induced diabetes mellitus.

Pancreas

The histopathological studies of the Pancreas of the slides of rats of Normal control group shows a large islet structure surrounded by exocrine gland tissue. There are no inflammatory cells are seen in the islet. The slides of diabetic control group shows the islet structure surrounded by exocrine gland tissue. Vacuolation of the islet cells and lymphocytic infiltration into the islet is seen. Glibenclamide (Standard antidiabetic drug) when administered at the dose level of 3 mg/kg b.w to rats of Group III showing a large islet structure surrounded by exocrine gland tissue. There are no inflammatory cells are seen in the islet. *Eriobotrya japonica* seeds when given at the dose level of 50 mg/kg b.w to rats of Group IV showed an islet structure with exocrine gland tissue seen at the upper edge. In this slide several inflammatory cells are seen in the islet and there is also vacuolation of islet cells present. When the extract is administered at the dose level of 100 mg/kg b.w to rats of Group V, it shows an islet structure surrounded by exocrine gland tissue. Few inflammatory cells are seen at the margins of the islet.

Liver

The histopathological examination of the liver slides of rats of Normal control group showed the portal triad area with no abnormality. Livers of the rats of diabetic group showed sinusoidal dilatation and necrosis with inflammatory cell infiltration and haemorrhage. Standard anti-diabetic drug Glibenclamide when given at the dose level of 3 mg/kg b.w to rats of Group III showed the portal triad area with no abnormality. *Eriobotrya japonica* seeds when given at the dose level of 50 mg/kg b.w to rats of Group IV showed the portal triad area with no abnormality. When the ethanolic extract was administered at the dose level of 100 mg/kg b.w to rats of Group V it showed the portal triad area with no abnormality.
HISTOPATHOLOGY OF PANCREAS IN RATS
DIABETES INDUCED BY STREPTOZOTOCIN (STZ)

Fig 1: Group I – Normal Control
Pancreas of rats showing a large islet structure surrounded by exocrine gland tissue. No inflammatory cells are seen in the islet (H&E x 40X).

Fig 2 (a): Group II – Diabetic Control
Pancreas from diabetic rats showing a islet structure surrounded by exocrine gland tissue. There is vacuolation of the islet cells and lymphocytic infiltration into the islet. (H&E x 40X).

Fig 2 (b): Group II – Diabetic Control.
Pancreas from diabetic rats showing a islet structure surrounded by exocrine gland tissue. There is vacuolation of the islet cells and lymphocytic infiltration into the islet. (H&E x 40X).

Fig 3: Group III–STZ*+Standard anti diabetic drug Glibenclamide (3 mg/kg b.w).
Pancreas from diabetic rats showing a large islet structure surrounded by exocrine gland tissue with no vacuolation. No inflammatory cells are seen in the islet (H&E x 40X).

Fig 4: Group IV– STZ*+Portulaca oleracea (50mg/kg b. w).
Pancreas from diabetic rats showing a large islet structure with exocrine gland tissue seen at upper edge. Few inflammatory cells are seen in the islet. (H&E x 40X)

Fig 5: Group V– STZ*+ Portulaca oleracea (100 mg/kg b.w).
Pancreas from diabetic rats showing a large islet structure with exocrine gland tissue seen at upper edge. No inflammatory cells are seen in the islet. (H&E x 40X)

*Streptozotocin (STZ) (50mg/kg) b.w. given once i.
HISTOPATHOLOGY OF LIVER IN RATS
DIABETES INDUCED BY STREPTOZOTOCIN (STZ)

Fig 6: Group – I Normal Control
Liver of rats showing the portal triad area. No. abnormality seen. (H&E x 40X)
BD= Bile Duct, PV= Portal Vein

Fig 7: (a) Group II- Diabetic Control
Streptozotocin (STZ) (50mg/kg) b.w.
Liver of diabetic rats showing the sinusoidal dilatation and necrosis with inflammatory cell infiltration & haemorrhage (H&E x 40X).

Fig 7(b): Group II- Diabetic Control
Streptozotocin (STZ) (50mg/kg) b.w.
Liver of diabetic rats showing inflammatory cell infiltration and haemorrhage around a bile duct. (H&E x 40X)

Fig 8: Group III- STZ* + Standard Antidiabetic Glibenclamide (3 mg/kg b.w)
Liver of diabetic rats showing the portal area. No. abnormality seen. (H&E x 40X)
BD= Bile Duct, HA= Hepatic Artery.

Fig 9: Group-IV- STZ* + Eriobotrya japonica seeds (50 mg/kg b.w)
Liver of diabetic rats showing the portal area. No. abnormality seen. (H&E x 40X)
BD = Bile Duct, PV = Portal Vein.

Fig 10: Group V- STZ* + Eriobotrya japonica seeds (100mg/kg b.w)
Liver of diabetic rats showing the portal area. No. abnormality seen. (H&E x 40X)
BD = Bile Duct, PV = Portal Vein.

* Streptozotocin (STZ) (50mg/kg) b.w. given once i.p
DISCUSSION
The primary organ of the body involved in sensing the organism’s dietary and energetic states through glucose concentration in the blood and in response to elevated blood glucose is pancreas where insulin is secreted. Alloxan and Streptozotocin has been the usual substance used for the induction of diabetes mellitus. It destroys the beta cells of the pancreas whereby it causes a massive reduction in insulin release. Insulin deficiency can lead to various metabolic alterations in the animals viz increased blood glucose and increased lipid profile.

Herbal plants have been of greater attention as an alternative to conventional therapy. The demand for these remedies has currently increased. Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products.[35-36]

The Indian indigenous drugs has great importance both from economic and professional point of view. A large number of plants have been reported to possess anti-diabetic activity e.g., Aconitum napeilus, Aloe vera, Carum carvi, Cichorium intybus, Allium cepa, Aralia cachemirica, Allium sativum, Momordia charantia.

Albino rats whose weight was in the range of 180-210 g were procured from IIM Jammu. They were kept in polypropylene cages under uniform conditions of food, water, temperature and degree of nursing care. It was ensured that the animals are in good health. Male and female rats were kept in separate cages so that there was no interference in evaluation of biochemical parameters. The temperature and the humidity were in the range of 15-25°C and 70-75% respectively.

The phytochemical screening of ethanolic extract of seeds of Eriobotrya japonica carried out by standard procedures revealed the presence of alkaloids, flavanoids and glycosides.

The results of the present study found that ethanolic extract of Eriobotrya japonica seeds have reduced the glucose level in animals made diabetic with streptozotocin. Streptozotocin has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of free radical damage caused by streptozotocin. In the present studies, ethanolic extract of Eriobotrya japonica seeds showed significant glucose lowering, hepatoprotective and hypolipidemic activity. The glucose lowering effect of the ethanolic extract may be due to the enhanced secretion of insulin from the beta cells of
pancreas or may be due to increased tissue uptake of glucose by enhancement of insulin sensitivity.

The literature reports reveal that flavonoids present in the ethanolic extract known to possess glucose lowering, hypolipidemic and hepatoprotective activity. Since many antidiabetic drugs do not correct dyslipidemia and hepatic damage, the observed hepatoprotective and hypolipidemic effects of the plant extract in diabetic rats makes *Eriobotrya japonica* seeds quite important in the management of diabetes. Since there is a strong well-established link between diabetes mellitus, dyslipidemia, obesity, hypertension and ischemic heart disease, effect of the ethanolic plant extract on weight loss/gain needs to be explored on scientific base.

**CONCLUSION**

The ethanolic extract of *Eriobotrya japonica* seeds has beneficial effects on blood glucose levels as well as improving hyperlipidemia and other metabolic aberrations. Further studies are needed on pharmacological and biochemical investigations which will clearly elucidate the mechanism of action and will help in projecting this plant as an therapeutic target in diabetics research. The level of morbidity and mortality related to this disease and its potential complications which are enormous, pose significant healthcare burdens on the families and society in India. There has shown tremendous increase in younger people. There is an urgent need for change the lifestyle of people. The inclusion of fruits and vegetables is important that will reduce the frequency of taking medicines in near future.

**ACKNOWLEDGEMENT**

Special thanks are to Sri Krishna Drugs Ltd., C-4 Industrial Area Uppal, Hyderabad for providing me a free gift pure sample of Glibenclamide which was used as standard anti diabetic drug and also to University Grants Commission for financial assistance for the work. The facilities which were provided by the Department of Pharmaceutical Sciences University of Kashmir for carrying out this work also need appreciation.

**CONFLICTS OF INTEREST:** NONE.
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