Original Research Article

A comparative study of antihyperglycemic effect of *Gymnema sylvestre* and metformin in streptozotocin induced diabetic rats

Sarita Mulkalwar, Aadil S. Shah*, Pallav Kataria, Tanya Gupta, A. V. Tilak, Bhagyashree Sharma

ABSTRACT

**Background:** Diabetes mellitus (DM) is a metabolic disorder that has the phenotype of hyperglycemia. According to World Health Organization (WHO) there were 65.1 million diabetics in India in 2013, International Diabetes Federation estimates this to increase to 190 million by 2035. Although a number of drugs are available for treatment of DM, their cost and safety profile are major concern. Medicinal plants are used by clinicians for treatment of diabetes. *Gymnema sylvestre* (GS) extract has been reported to increase insulin levels in diabetic rats. This study was designed to compare the antihyperglycemic effect of *Gymnema sylvestre* with metformin.

**Methods:** Diabetes was induced in Sprague-Dawley rats using streptozotocin 45mg/kg. Methanolic extract of *Gymnema sylvestre* 120mg/kg p.o. prepared using Soxhlet apparatus.

**Results:** GS extract reduced blood glucose levels but not statistically significant. GS extract increased HDL and triglycerides, reduced both serum ALT and AST but no statistical significance seen. Metformin significantly increased serum urea, which was not seen in GS extract group. GS extract showed regenerative changes in pancreas, liver and kidney.

**Conclusions:** The study investigation demonstrates that methanolic extract of GS possesses antihyperglycemic and hypolipidaemic activity and so it can be considered as a promising natural remedy in a prediabetic state and in mild hyperlipidemia to prevent its progression. Increase in β cell regeneration activity could be a probable mechanism of action. However, further long term clinical studies are recommended to define its possible role in diabetes mellitus and hyperlipidemia. Role of GS as a potential hepatoprotective agent also needs further evaluation.

**Keywords:** Antihyperglycaemic, Beta cell regeneration, *Gymnema sylvestre*, Hepatoprotective, Hypolipidemic

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that has the phenotype of hyperglycemia. According to World Health Organization (WHO) there were 65.1 million diabetics in India in 2013, International Diabetes Federation estimates this to increase to 190 million by 2035, making India second largest with disease burden and accounting approximately 20% of world diabetic population. The fact that there is shift in age of onset to younger age groups is alarming, as this could have adverse effects on nation’s economy.1,3

Although a number of drugs are available for treatment of DM, their cost and safety profile are major concern.4

Due to recent trends in changing lifestyle and food habits, prediabetes is a rising concern. Lifestyle modification is the best action to prevent the progression of prediabetes into diabetes. But, being asymptomatic, patients of
prediabetes tend to neglect the advice of lifestyle modification. Many people prefer ayurvedic medication over allopathic drugs due to their claim of being side-effect free.

Medicinal plants are used by clinicians for treatment of diabetes. Gymnema sylvestre (GS) extract has been reported to increase insulin levels in diabetic rats. This study was designed to compare the antihyperglycemic effect of Gymnema sylvestre with metformin.

Aim was to study the effect of Gymnema sylvestre on blood glucose levels in Sprague-Dawley rats and compare it with metformin.

**Objectives**

- To study the effect of Gymnema sylvestre extract on blood glucose levels in Sprague-Dawley rats.
- To compare the effect of Gymnema sylvestre extract with metformin on blood glucose levels in Sprague-Dawley rats.
- To study the mechanism of action of Gymnema sylvestre by estimating serum C-peptide levels and pancreatic histopathology in Sprague-Dawley rats.
- To study the effects of Gymnema sylvestre extract on lipid profile of Sprague-Dawley rats.
- To study the effects of Gymnema sylvestre extract on liver and renal functions of Sprague-Dawley rats.

**METHODS**

The study was conducted after taking approval from Institutional Animal Ethics Committee (IAEC) of Dr. D. Y. Patil Medical College, Pimpri, Pune. Study was performed following the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. 32 Sprague-Dawley rats of either sex weighing 150-250gm were included in the study. Animals were fed with commercially available ‘Nutrimix Std - 1020’ manufactured by Baramati Agro Ltd, acquired from Nutrivet Life Sciences, Pune. Drinking tap water supplied by Pimpi-Chinchwad Municipal Corporation was provided to the rats through the feeding bottles. Both food and water were available to rats ad libitum. Animals were housed in groups of five in a standard big polypropylene cage measuring 40X27.5X13.5cm, having wire mesh top with provision for drinking water and space for pellets. Corn cob was used as padding material in the cages. Animals were kept and maintained under laboratory conditions of temperature (25°C±5°C), relative humidity (55±10%), and 12-hour light/day cycle.

Streptozotocin (STZ) was obtained from Sisco Research Laboratories Pvt. Ltd, MIDC, Taloja, Maharashtra. Metformin were obtained from Research-Lab Fine Chem Industries, Mumbai, Maharashtra. Commercial preparation of Gymnema sylvestre powder was obtained from Manakarnika Aushadhalaya, Chinchwadgaon, Pune.

Methanolic extract of Gymnema sylvestre was preferred as it yields maximum amount of gymnemic acid compared to ethanolic and aqueous extracts; which is the proposed active ingredient for antihyperglycemic action. The methanolic extract was prepared at Dr. D. Y. Patil College of Ayurveda and Research Centre, Pimpri, Pune, Maharashtra. Soxhlet apparatus was used for preparing methanolic extract of Gymnema sylvestre. Accu-Chek Active (Roche Products Pvt. Ltd.) was used for measuring BSL by tail prick method.

Diabetes was induced in rats of either sex of 8 to 10 weeks age. Streptozotocin 45mg/kg dissolved in 1ml distilled water was given intraperitoneal to overnight fasted rats. On 3rd day of injection BSL level was checked using glucometer. Animals having BSL more than 350mg/dl were included in the study and divided into three groups of 8 rats each. Normal control group received 1ml distilled water intraperitoneally, without STZ.

<table>
<thead>
<tr>
<th>Group</th>
<th>Induction of diabetes</th>
<th>Study drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Normal)</td>
<td>Not induced</td>
<td>Distilled water 1ml p.o.</td>
</tr>
<tr>
<td>Group 2 (Diabetic Control)</td>
<td>STZ 45mg/kg</td>
<td>Distilled water 1ml p.o.</td>
</tr>
<tr>
<td>Group 3 (Gymnema sylvestre)</td>
<td>STZ 45mg/kg</td>
<td>Gymnema sylvestre extract 120mg/kg p.o.</td>
</tr>
<tr>
<td>Group 4 (Metformin)</td>
<td>STZ 45mg/kg</td>
<td>Metformin 90mg/kg in 1ml Distilled water p.o.</td>
</tr>
</tbody>
</table>

At the end of the study all the animals were taken to APT Research Foundation, Vadaon khurd, Sinhagad Road, Pune, Maharashtra. They were sacrificed by cervical dislocation after anaesthetising using chloroform. Blood samples were collected by cardiac puncture and analysis was done.

Histopathology of pancreas, liver and kidney was done of representative samples of Diabetic Control, Gymnema sylvestre and Metformin groups.

Serum C-peptide was measured using Fully Automated Chemi Luminescent Immuno Assay.

All values of results are presented as mean ± standard error of mean (SEM). The statistical analysis for confirming induction of diabetes was evaluated by Paired Student’s t-test. One-way analysis of variance (ANOVA) followed by Tukey’s test was used for statistical comparison between control and various treated groups. Statistical significance was accepted at P <0.05 value. Data analysis was done using Primer for Biostatistics version 5.0.
RESULTS

Paired student’s t-test was applied to blood glucose levels before induction and on 3rd day after STZ injection of 24 rats. There was significant increase in glucose level in all the rats after STZ injection. Diabetes was induced in all rats by STZ 45 mg/kg dose.

Table 2: Induction of diabetes.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Mean±SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (8)</td>
<td>102.9±4.27</td>
<td>-----</td>
</tr>
<tr>
<td>Diabetic Control (6)</td>
<td>460±24.18</td>
<td>&lt;0.05 @</td>
</tr>
<tr>
<td>Gymnema sylvestre 120mg/kg (6)</td>
<td>383.7±53.86</td>
<td>Not significant</td>
</tr>
<tr>
<td>Metformin 90mg/kg (7)</td>
<td>231±40.95</td>
<td>&lt;0.05* &lt;0.05#</td>
</tr>
</tbody>
</table>

Mean blood glucose level was significantly increased in diabetic control group when compared to normal. There was significant reduction in mean blood glucose of metformin control group when compared to diabetic control and Gymnema sylvestre extract. Although Gymnema sylvestre extract reduced mean blood glucose level there was no significant difference when compared to diabetic control group.

Table 3: Blood glucose levels (mg/dl) (8th week).

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Mean±SEM</th>
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<td>Metformin 90mg/kg (7)</td>
<td>231±40.95</td>
<td>&lt;0.05* &lt;0.05#</td>
</tr>
</tbody>
</table>

@ → when compared to Normal
* → when compared to Diabetic control
# → when compared to Test Drug

Serum C-peptide levels (8th week)

Serum C-peptide levels was 6.89±1.61 mg/ml in normal rats. In diabetic group C-peptide levels were not detected (<0.05 mg/ml) as expected. In metformin and Gymnema sylvestre groups C-peptide levels of not all rats were detectable. Three rats in metformin group had C-peptide levels (ng/ml) 0.05, 0.054, 0.057 and the blood glucose levels (mg/dl) were 296, 150, 132 respectively. Two rats in Gymnema sylvestre group had C-peptide (ng/ml) 0.08, 0.059 and blood glucose levels (mg/dl) were 144, 367 respectively. Correlation between serum C-peptide levels and blood glucose levels could not be established due to small sample size.

Lipid profile (8th week)

Serum cholesterol was increased in both metformin and Gymnema sylvestre groups compared to diabetic group, although there was no statistical significance. Serum cholesterol levels were significantly reduced in diabetic group when compared to normal rats.

Serum triglycerides were significantly increased in diabetic control group compared to normal rats. Triglycerides were increased in Gymnema sylvestre group compared to diabetic control, although the difference was not statistically significant. Triglycerides were reduced in metformin group compared to diabetic group, but the difference was not statistically significant. Also, triglycerides in metformin group were lower than Gymnema sylvestre group, there was no statistical significance.

Table 4: Lipid profile (8th week).

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Serum cholesterol (mg/dl) Mean±SEM</th>
<th>Serum Triglycerides (mg/dl) Mean±SEM</th>
<th>Serum HDL (mg/dl) Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (8)</td>
<td>81.2±2.1</td>
<td>67.6±1.7</td>
<td>32.8±1.1</td>
</tr>
<tr>
<td>Diabetic Control (6)</td>
<td>59.33±2.93*</td>
<td>155.7±9.54*</td>
<td>16.45±0.40*</td>
</tr>
<tr>
<td>Gymnema sylvestre 120mg/kg (6)</td>
<td>66.83±4.47*</td>
<td>184±41.76</td>
<td>17.8±1.31</td>
</tr>
<tr>
<td>Metformin 90mg/kg (7)</td>
<td>60.86±3.62</td>
<td>114.7±8.82</td>
<td>18.04±0.72</td>
</tr>
</tbody>
</table>

* → p <0.05 when compared to Normal

Serum transaminases (8th week)

There was no significant difference in serum ALT between normal rats and diabetic control. Serum ALT levels were reduced in both metformin and Gymnema sylvestre groups compared to diabetic control, more so in metformin group; but the differences were not statistically significant.

Serum AST levels were significantly increased in diabetic control group compared to normal rats. These levels reduced in both metformin and Gymnema sylvestre groups. The reduction was statistically significant in Gymnema sylvestre group when compared to diabetic group but not when compared to metformin group. Metformin did not cause statistically significant reduction in AST levels when compared with diabetic group.

Serum urea and creatinine (8th week)

Serum urea levels were comparable in both normal and diabetic control groups. Gymnema sylvestre reduced serum urea levels when compared to diabetic control, but the difference was not statistically significant. Metformin
increased urea levels but not significantly when compared to diabetic group; although the increase was statistically significant when compared to normal rats.

Serum creatinine levels were comparable in both normal and diabetic groups. Both metformin and Gymnema sylvestre did not cause any significant change in serum creatinine levels.

Table 5: Serum transaminases (8th week).

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Serum ALT (U/L) Mean±SEM</th>
<th>Serum AST (U/L) Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (8)</td>
<td>50.04±11.59</td>
<td>130.8±4.33</td>
</tr>
<tr>
<td>Diabetic Control (6)</td>
<td>51.43±1.90</td>
<td>173.7±6.07*</td>
</tr>
<tr>
<td>Gymnema sylvestre</td>
<td>48.05±4.07</td>
<td>127.8±9.37#</td>
</tr>
<tr>
<td>120mg/kg (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>46.15±5.45</td>
<td>148.4±9.04</td>
</tr>
<tr>
<td>90mg/kg (7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p <0.05 when compared to Normal
# p <0.05 when compared to Diabetic Control

Table 6: Serum urea and creatinine (8th week).

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Serum urea (mg/dl) Mean±SEM</th>
<th>Serum creatinine (mg/dl) Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (8)</td>
<td>38.49±3.84</td>
<td>1.31±0.13</td>
</tr>
<tr>
<td>Diabetic Control (6)</td>
<td>58.55±3.95</td>
<td>1.417±0.35</td>
</tr>
<tr>
<td>Gymnema sylvestre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120mg/kg (6)</td>
<td>56.53±5.50</td>
<td>2.2±0.45</td>
</tr>
<tr>
<td>Metformin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90mg/kg (7)</td>
<td>61.18±8.38*</td>
<td>1.343±0.34</td>
</tr>
</tbody>
</table>

* p < 0.05 when compared to Normal

Histopathological observations

Pancreas

Pancreatic histopathology of Gymnema sylvestre group showed normal islet with adequate β cell histomorphology. Comparatively, in diabetic group there were atrophic and focal necrotic changes seen. Metformin group showed focal atrophic changes with loss of β cells in islets. This is similar to what observed in previous studies and are in line with the proposed β cell regeneration property of Gymnema sylvestre (Figures 1, 2 and 3).

Liver

Both Gymnema sylvestre and metformin had normal hepatic parenchyma with normal portal vascular tissue, with the absence of any metabolic or inflammatory changes. Degenerative changes were seen of hepatocyte with focal congestion of portal vessel (Figures 4, 5 and 6).
Histopathology in diabetic control group show interstitial haemorrhage in renal parenchyma with focal degenerative changes (Figures 7,8 and 9).

Figure 4: Liver in diabetic control group showing focal congestion and focal degenerative changes of hepatocytes (H and E, X100).

Figure 7: Kidney in diabetic control group showing congestion and interstitial hemorrhages and focal degenerative changes in renal tubules (H and E, X100).

Figure 5: Liver in Gymnema sylvestre group showing normal hepatic parenchyma with intact nucleus and cell borders (H and E, X100).

Figure 8: Kidney in Gymnema sylvestre group showing normal histomorphology of glomeruli and renal tubules (H and E, X100).

Figure 6: Liver in metformin group showing normal hepatic parenchyma with intact nucleus and cell borders (H and E, X100).

Figure 9: Kidney in metformin group showing normal histomorphology of glomeruli and renal tubules (H and E, X100).

Kidney

Both Gymnema sylvestre and metformin groups had normal histomorphology of glomeruli and renal tubules without any degenerative or inflammatory changes.

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DISCUSSION

Diabetes mellitus is cause for global health concern as the disease is rapidly progressing, also the age of onset to younger age groups is alarming. The standard drug therapy has various side-effects and hence need for development of new drugs with better safety profile. Many medicinal plants are used by traditional medicine for treatment of diabetes. Gymnema sylvestre leaves when chewed have property of paralyzing the sense of taste for sweet for short period.

The treatment of prediabetic patients is mainly lifestyle modification in the form of weight reduction, exercise and diet control. Lifestyle education at regular health check-up for people with prediabetes lower progression to diabetes by reducing modifiable risk factors. But to follow these lifestyle modifications requires motivation and physicians should assess patient’s readiness to work towards change. Studies have shown that people are resistant to lifestyle change. Looking at the increasing number of people in prediabetic stage, there is an urgent need to explore different therapeutic options. Since people in India prefer alternative medicine due to their claim of being side effect free, the exploration of vast knowledge of Ayurvedic medicine can help us in understanding their role in various chronic diseases either to prevent further development or to prolong the onset.

Kumar et al, studied the effect of ethanolic extract of Gymnema sylvestre on STZ induced diabetic rats. They found that there was significant reduction in blood glucose levels.

Kumar et al, also studied the effect of ethanolic extract of Gymnema sylvestre on High Fat Diet induced diabetic rats. GS extract significantly reduced glucose levels in diabetic rats. GS extract has significantly reduced the levels of serum leptin and serum insulin compared to diabetic group. This suggest that GS is able to decrease the insulin resistance, which contributes to its antihyperglycemic action. GS extract had no effect on blood glucose level and lipid profile in non-diabetic rats.

Daisy P et al, concluded that long term administration of GS extract results in significant reduction of blood glucose levels.

Shanmugasundaram KR et al, studied the effect of GS extract in alloxan induced rabbits. They found that GS extract significantly reduced the blood glucose levels after 12 weeks when compared to diabetic rats. They further continued the study and at 24 weeks of GS extract administration the glucose levels were near normal.

Shanmugasundaram ERB et al, conducted a clinical study on 27 patients of Type 1 diabetes on insulin. Patients were given 400mg/kg/day GS aqueous extract. After 10-12 months of add-on GS extract, the blood glucose levels were reduced and there was reduction in patient’s insulin requirement.

Baskaran K et al, studied the effectiveness of GS leaf extract on 22 Type 2 diabetic patients on conventional oral antihyperglycemic agents. GS extract was administered for 18-20 months as a supplement to the conventional drugs. Patients showed significant reduction in blood glucose. Five patients were able to discontinue their conventional drugs and dosage of conventional drugs in other patients were reduced.

Sugihara Y et al, investigated the antihyperglycemic action of methanolic extract of GS in STZ induced diabetic mice. The study concluded that GS causes significant reduction in glucose levels.

In this study GS extract reduced blood glucose levels, but the difference was not statistically significantly. The short duration of study and also the dose of GS extract could have been the limiting factors in this study. Hence, future studies of longer duration and increasing dose are needed to decide the effectiveness of GS extract in diabetes.

Effect of acetone extract of GS was studied in Daisy P et al, which showed that the extract increases the secretion of insulin in STZ-induced diabetic rats.

Liu B et al, documented that aqueous extract of GS has insulin secretagogues action in-vitro on MIN6 β-cell line as well as human islets of Langerhans. Al-Romainian A et al, studied the effect of long term administration of GS extract, Om Santal Adivasi (OSA), on patients of Type 2 diabetes mellitus. They found that OSA significantly increased circulating C-peptide and insulin levels which were associated with reduced blood glucose levels. They also demonstrated the stimulatory effect on OSA on isolated human islets of Langerhans resulting in increased insulin secretion.

Al-Romainian et al, Ahmed ABA et al, and several studies have suggested the possible mechanism of antihyperglycemic action of GS extract could be pancreatic β cell regeneration, secretion of more insulin from pancreas. β cell regeneration was seen in histopathology of pancreas of GS extract treated group rats.

Sugihara Y et al, found that reduction in glucose levels by GS extract were accompanied by an increase in serum insulin levels.

Baskaran K et al, found that GS supplementation raised the serum insulin levels in patients of type 2 diabetes on conventional oral antihyperglycemic agents.

Various in-vivo and in-vitro studies have proven that GS extract significantly increases insulin secretion. In this study, serum C-peptide levels were increased in few rats receiving GS extract, however statistical test could not
be applied. This could be due to the short duration of the study.

Gymnema sylvestre had shown beneficial effects on lipid profile in rats receiving High Fat Diet. There was significant reduction in TC, TGs, LDL-C, VLDL-C and increase in HDL-C levels in various previous studies.\textsuperscript{10,11}

Daisy P et al, studied the effects of GS acetone extract on STZ induced diabetic rats. They found that long term administration of extract reduced TC, LDL, VLDL levels.\textsuperscript{12}

Kumar DS et al, compared the hypolipidaemic action of \textit{Gymnema sylvestre} with Atorvastatin. GS extract showed significant reduction of TGs, TC, LDL-C, VLDL-C and increase in HDL-C levels in a dose dependent manner. GS extract 200mg/kg having better effect than GS extract 100mg/kg. Both the doses were however inferior to Atorvastatin 10mg/kg.\textsuperscript{20}

Shanmugasundaram ERB et al. conducted a clinical study on 27 patients of Type 1 diabetes on insulin. They found that serum lipids returned to near normal after 10-12 months of treatment.\textsuperscript{14}

In this study, there was no significant effect of GS extract on the lipid profile. Serum HDL levels did increase but they were not statistically significant. This might be due to the short duration of GS administration. Effect of chronic administration of the extract should be studied to evaluate its beneficial effect on lipid profile.

Shanmugasundaram et al, also showed that there was significant reduction of ALT and AST by GS extract when compared to diabetic group.\textsuperscript{13}

In this study, the authors found that there was reduction in AST and ALT but it was not statistically significant. Histopathology report have also shown normal hepatic parenchyma in GS extract treated group. This suggest that GS extract might have some hepatoprotective effect. Further studies are needed to evaluate this effect of GS.

GS extract also reduces the absorption of glucose from intestine. GS bind to Na\textsuperscript{+}-glucose symport in intestine and prevent absorption of glucose, thus reducing post-prandial blood glucose levels.\textsuperscript{21}

Chen G and Guo M conducted an in-vitro study to understand the mechanism of GS. GS exhibited significant inhibitory activity against α glucosidase. They compared the inhibitory activity of GS to acarbose. They found that GS has α glucosidase inhibitor property which was statistically significant when compared with acarbose. The IC\textsubscript{50} of GS was found to be 68.70±1.22µg/ml.\textsuperscript{22}

Metformin had significantly increased the levels of serum urea compared to normal non-diabetic rats. Impaired renal function has major effect on the dosing of metformin. It is advised to assess renal function before the starting metformin and to monitor function annually.\textsuperscript{23} GS extract reduced the levels of serum urea, but it was not statistically significant. GS extract also leads to increase in serum creatinine levels, but the difference was not significant. Histopathology report shows a normal glomeruli and renal tubules. There might be some protective effect of GS on kidney, but further studies are required to establish its effect in patients of diabetes with renal impairment.

Patients of metabolic syndrome are required to do lifestyle modification. Many patients are not able to strictly follow these lifestyle changes. Initiating pharmacotherapy in these patients is not always an option due to the side-effect profile of the conventional antidiabetic and antihyperlipidemic drugs. GS extract due to its beneficial effect on blood glucose and lipid profile can be used in these patients.

**CONCLUSION**

Ayurveda practice continues today to treat various chronic human diseases and provides positive health benefits to the people and plays significant role in prevention of various diseases. Our investigation demonstrates that methanolic extract of GS possesses antihyperglycemic and hypolipidaemic activity and so it can be considered as a promising natural remedy in a prediabetic state and in mild hyperlipidaemia to prevent its progression. It can also be used as an adjuvant treatment along with the standard allopathic treatment to treat diabetes and hyperlipidaemia. Increase in β cell regeneration activity could be a probable mechanism of action. However further long term clinical studies are recommended to define its possible role in diabetes mellitus and hyperlipidaemia. Role of GS as a potential hepatoprotective agent also needs further evaluation.

**ACKNOWLEDGEMENTS**

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the Institutional Animal Ethics Committee (IAEC) of Dr. D. Y. Patil Medical College, Pimpri, Pune, India

**REFERENCES**


