

# AN IMMUNOHISTOCHEMICAL EVALUATION OF GLUT 2 IN ISLETS OF LANGERHANS TREATED WITH GYNEMA SYLVESTRE AND METFORMIN IN STREPTOZOTOCIN-INDUCED ANIMALS

## Abstract

Diabetes mellitus is a generally known metabolic disorder affecting individuals, not only the elderly population, but all age groups. Among the etiologies of diabetes, reduction of glucose transporter GLUT-2 is accompanied by a loss in glucose-mediated insulin secretion, causing diabetes. To treat diabetes, the present scenario is the demand for time to use herbal drugs with minimal detrimental effects. Considering the benefits of *Gymnema sylvestre*, an anti-diabetic herbal drug, our current study is designed to expose the plausible relationship between the anti-diabetic activity and GLUT-2 expression, and the efficacy is evaluated in a streptozotocin-induced diabetic model. **Materials and Methods:** Wistar albino adult rats (n=36) weighing about 140–160 g. Each group consisted of 6 animals and was divided into six groups based on the high fat diet (42 days), the streptozotocin-induced diabetic model (5 days) and treated for 22 days using ethanolic extract of *Gymnema sylvestre* at low (200mg) and high dosage (400mg) and metformin (25mg). During the experimental period, blood glucose levels and the animal weight were carefully monitored. **Results:** Decreased body weight, pancreatic weight, and increased blood glucose were observed in Group III along with a reduced level of GLUT-2 expression, indicating the manifestation of diabetes. In diabetic rats, however, these abnormalities are significantly restored after treatment with *Gymnema sylvestre*, albeit at a higher dosage. The *Gymnema sylvestre* displays the antidiabetic activity through the regeneration of pancreatic  $\beta$ -cells, maintaining GLUT-2 expression in diabetic induced animal model and Our histological study of pancreatic tissue also confirms the regeneration of beta cells.

**Keywords:** Type-2 diabetes, *Gymnema Sylvestre*, GLUT-2, Immunohistochemistry.

## **Introduction:**

Diabetes mellitus is the most common metabolic disorder. It is characterized by hyperglycemia that results from an insulin deficiency and it is associated with complications affecting the eye, heart, kidney, and nerves (Punthakee et al., 2018). Diabetes is a fast growing problem in India. In the year 2000, India had 31.7 million people affected by diabetes, followed by China (20.8 million) and the United States (17.7 million), respectively. According to Wild et al. (2004), it is predicted that by 2030, diabetes will affect 79.4 million people. The estimation of diabetes in 2019 showed about 77 million people had diabetes in India, which is expected to increase as 134 million will by 2045 (Pradeepa et al. 2021).

Diabetes mellitus is classified into Type 1 and Type 2. Type 1 diabetes is an autoimmune disease characterized by an inflammatory reaction around the islets of Langerhans followed by specific destruction of  $\beta$ -cells (Tosone et al., 2013). Type 2 diabetes is characterized by the occurrence of peripheral insulin resistance and impaired insulin secretion (Mahler and Adler, 1999). Diverse etiologies intricate the causes of diabetes, among which GLUT is an important biochemical entity involved in the development of diabetes. GLUT-2 is a family of glucose transporters restricted to the plasma membrane of insulin-positive beta cells of adults and other tissues such as the liver, kidney, and intestinal mucosal epithelium (Jorns et al. 1995). GLUT-2 regulates the transportation of glucose into the pancreatic cell and thus promotes insulin secretion, and the reduction of GLUT-2 is correlated with a loss in glucose-induced insulin secretion, causing diabetes (Mueckler et al., 1994; Olli Laukkanen et al., 2005). GLUTs facilitate the passive transport of glucose across the plasma membrane (Mueckler & Thorens B 2013)

Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to their fewer side effects (Pritesh Patel et al., 2012). High doses may lead to side effects including hypoglycemia, weakness, shakiness, excessive sweating, and muscular dystrophy (Tiwari et al., 2014). Gymnemic acid may be hepatotoxic at a higher dose in mice (Gholap et al., 2005). Therefore, the present situation demands the use of herbal drugs from plant sources to treat such ailments with minimal detrimental effects. *Gymnema Sylvestre* commonly known as Sirukurunjan (Tamil), is a very popular anti-diabetic plant traditionally used as a sugar destroyer (Najafi and Deokule, 2011) in India for centuries. This herbal drug is also used for many ailments such as jaundice (Di Fabio et al., 2013), cardiopathy (Kumar et al., 2012), asthma, bronchitis (Pragya Tiwari et al., 2014), appetite suppressant (Sujin RM. 2008),

and conjunctivitis (Parijat Kanetkar et al., 2007) and other diseases (David and Sudarsanam 2013; Rongzi Li et al., 2019).

*Gymnema sylvestre* is widely used to treat diabetes and has been validated scientifically. However, the molecular mechanism supporting the hypoglycemic and anti-diabetic properties remains unclear. Furthermore, many studies have reported antidiabetic properties of *Gymnema sylvestre* (Prakash et al., 1986; Baskaran et al., 1990; Shanmugasundaram et al., 1990), but only a few reports are available for *Gymnema sylvestre* and GLUT-2 in the diabetic model. Thus, considering the benefits of *Gymnema Sylvestre*, the current study is designed to elucidate the plausible link between the anti-diabetic activity of *Gymnema Sylvestre* and GLUT-2 expression, and the efficacy is evaluated in a streptozotocin-induced diabetic model.

## **MATERIALS**

### **Plant material:**

The leaves of *Gymnema sylvestre* were collected from Chidambaram and the plant material was properly identified and confirmed with plant authentication number PARC/2018/3985. The powdered leaves were light green in colour. About 100 grams of powdered leaves were given to the pharmacy institute in Melmaruvathur to take the ethanolic extraction of *Gymnema sylvestre* through soxhlet apparatus.

### **Preparation of Ethanolic Extract of *Gymnema Sylvestre*:**

100g of dry leaf powder was packed into a soxhlet thimble and extracted with 80% ethanol. Then 80 % of ethanol is added until the powder is exhausted. The extraction procedure was carried out for 48 hours until the ethanolic extract was obtained. The collected solution from the flask was filtered through Whatman No. 1 filter paper. The solvent was evaporated under pressure at 90 °C by a rotary evaporator and the extract was stored at -20 °C in a freezer until further use.

## **METHODS:**

Adult Wister albino rats weighing 140–160 g were separated, housed in polypropylene cages, and kept under standard housing conditions of a 12:12 hr day-night cycle, 22–25 °C temperature, and 50–60% relative humidity. The animals were fed with standard rat pellets and were allowed to drink water ad libitum.

### **Experimental Plan:**

Wistar albino adult rats (n=36) were used for this study weighing about 140–160 g. Each group consisted of 6 animals and was divided into Group I, which was a control animal. **In Group II**, animals were fed a high-fat diet (67.5% lard oil, 31% cholesterol, 1% d1-methionine, 0.3% yeast powder, and 0.1% NaCl) for 42 days. **Group III**, animals were fed a high-fat diet for 14 days, followed by multiple-dose streptozotocin (40 mg/kg b.w.) administered intraperitoneally for 5 successive days to establish a Type 2 diabetic model. The Group IV and V were type 2 diabetic induced animals with streptozotocin then treated with ethanolic extract of *Gymnema sylvestre*. The extract is administered orally with gavages. **Group IV** received a low dosage of around 200 mg/kg b.w. and **Group V** received a high dosage of about 400 mg/kg b.w. for 23 days, respectively. **Group VI** is a positive control group of animals that were induced diabetic with streptozotocin and given Metformin (25 mg/kg b.w. for 23 days). During the experimental period, blood glucose levels and the animal weight were carefully monitored. The glucose level was diagnosed by collecting a blood sample from the tail vein (Fig.1) and determining it by using a glucose analyzer with a glucose strip inserted into the glucometer (Fig.2).



**Fig 1 Sample collection from tail vein**



**Fig 2 Glucometer with glucose strip**

### **Tissue Harvesting**

Animals were injected intraperitoneally with an overdose of 85 mg of ketamine and 15 mg of Xylazine/kg of body weight. At the end, organs such as the liver and pancreas were dissected out and weighed immediately after the sacrifice to determine the change in the weight of the organs with respect to their body weights. The harvested tissues were post-fixed in 10% formaldehyde and

processed for standard histology and immunohistochemistry. Sections were taken at a 5  $\mu\text{m}$  thickness and the sections were stained using the Harris hematoxylin and eosin method. (Bancroft 2012).

### **Immunohistochemistry**

For detection of GLUT-2 expression, the sections were deparaffinized, rehydrated, and subjected to 1%  $\text{H}_2\text{O}_2$  in PBS to quench the endogenous peroxidase activity, followed by blocking buffer (5% normal chicken serum in PBS and 0.3% Triton X-100) treatment at 4°C to minimize non-specific expression. Then the sections were incubated with a 1:100 dilution of human anti-GLUT-2 monoclonal antibody (CUSABIO TECHNOLOGY LLC, USA) overnight at 4°C. After washing with PBS, tissues were incubated with a 1:400 dilution of biotinylated goat anti-mouse secondary antibody for 20 min at 42°C. Subsequently, sections were treated with an avidin-biotin-peroxidase complex for 2 hours. The peroxidase activity was visualized using a stable diaminobenzidine solution and counterstained with hematoxylin. The GLUT-2 expressions were examined using a compound light microscope (Olympus CX31) and these results were quantified using the Image Analysis J 1.46 software (National Institutes of Health, Bethesda, Maryland, USA).

### **Statistical Analysis:**

Microsoft Excel (Version 2003) and SPSS (SPSS, Version 25, Inc., IBM) software were used to analyze the data. The values were expressed as the mean and standard error of the mean. A one-way ANOVA was performed in SPSS. The level of significance was determined with a "Tukey's post hoc" test and  $P < 0.05$  was considered as statistically significant.

## **Results:**

### **Animal Body weight**

In this present investigation, the animals in group II showed increased body weight ( $P < 0.05$ ) after the supplementation of a high-fat diet. The group III animals administered with streptozotocin-induced diabetes showed a significant reduction in body weight ( $P < 0.001$ ) when compared to control animals (Group I). The Group IV and Group V animals maintained their body weight after treatment of *Gymnema sylvestre* at low and high dosages compared to the Group III. These observations are

very similar to those made in streptozotocin-induced diabetic animals treated with metformin [Table 1].

**Table 1 Animal Body weight in grams**

Groups	14 <sup>th</sup> day	19 <sup>th</sup> day	28 <sup>th</sup> day	42 <sup>nd</sup> day
Group I	169.50 ± 1.94	172.50 ± 1.82	182.67 ± 1.36	204.83 ± 2.01
Group II	178.17 ± 1.2	186.33 ± 1.31	209.17 ± 2.19 @*	232.50 ± 1.53 @*
Group III	176.33 ± 1.11	148.17 ± 1.28 @***	105.67 ± 1.58 @***	88.67 ± 1.14 @***
Group IV	175.33 ± 1.49	147.67 ± 1.57 @***	164.67 ± 3.51 \$***	176.50 ± 3.89 \$***
Group IV	174.33 ± 1.62	149.83 ± 1.34 @**	171.83 ± 2.00 \$***	185.83 ± 2.38 \$***
Group VI	175.33 ± 1.46	147.33 ± 1.75 @***	177.50 ± 2.46 \$***	194.67 ± 1.57 \$***

Table 1 illustrates body weight of control and various groups during experimental period and value are presented as Mean ± SEM (n=6 animals each) with \* P<0.05, \*\* P<0.01 and \*\*\* P<0.001. @ - compared with control; \$ - compared with streptozotocin-induced diabetic animals (Group III).

### Liver and Pancreas Weight

The weight of the pancreas and liver [Graph 1 and 2] in Group II was highly elevated after the supplementation of a high-fat diet [Table 2]. While in Group III i.e., streptozotocin-induced diabetic animals, the liver weight was decreased to some extent, whereas the pancreas weight was significantly (P<0.05) reduced. Whereas *Gymnema sylvestre* treatment significantly increased pancreatic weight in Group IV and Group V animals at both dosages (200 mg and 400 mg), there was a marginal decrease in Group IV that did not show statistical significance. The Metformin administered animals (Group VI) did not show any noticeable changes in liver and pancreas weight, and these values were close to control animals.

**Table 2 Weight of the pancreas and liver immediate after the sacrifice in grams**

Groups	Pancreas	Liver
Group I	0.423 ± 0.018	5.31 ± 0.128
Group II	0.566 ± 0.023	6.54 ± 0.222
Group III	0.222 ± 0.008 @*	4.86 ± 0.170
Group IV	0.293 ± 0.022	5.20 ± 0.208
Group IV	0.332 ± 0.018	5.23 ± 0.090
Group VI	0.362 ± 0.020	5.26 ± 0.156

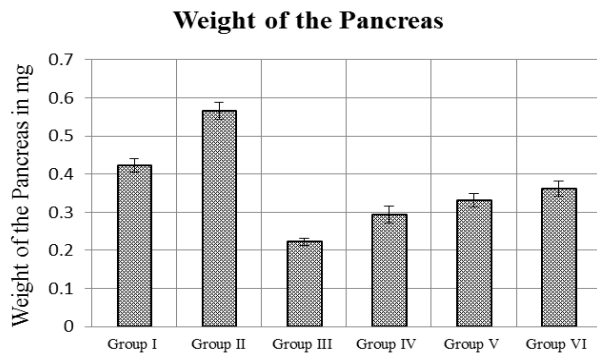
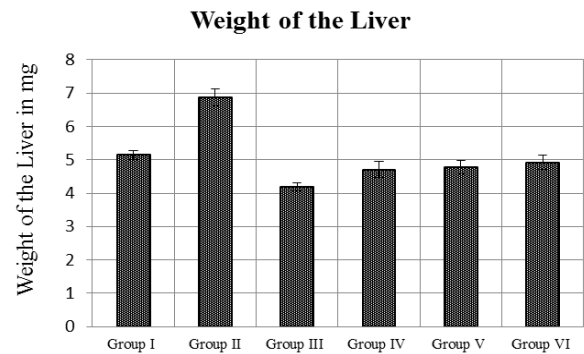
**Graph 1****Graph 2**

Table 2 and Graph 1 & 2 displays organ weight of pancreas and liver of different experimental groups. The values are stated as Mean  $\pm$  SEM of 6 animals each; Significance at \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ . @ - comparison of all experimental groups with control.

### Blood glucose levels

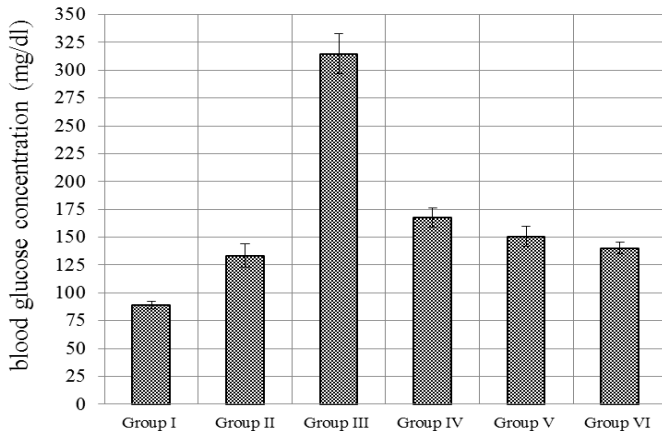
Blood glucose levels [Graph 3] were significantly elevated ( $P < 0.001$ ) in Groups III compared to control animals (Group I). In streptozotocin-induced diabetic animals (Groups V and VI), administration of *Gymnema sylvestre* (400mg) and metformin resulted in a decrease in blood glucose levels. Though this level was decreased at low dose *Gymnema sylvestre* administration in group IV, the improvement was noticeable at high dosage. The Group II rats fed with high fat did not show any significant shifts in their blood glucose levels. The data for blood glucose level was shown in Table 3.

**Table 3 Blood Glucose Level**

Groups	14 <sup>th</sup> day	19 <sup>th</sup> day	28 <sup>th</sup> day	42 <sup>nd</sup> day
Group I	89.83 $\pm$ 2.79	91.33 $\pm$ 2.85	101.67 $\pm$ 4.3	89 $\pm$ 3.3
Group II	84.67 $\pm$ 2.5	129.50 $\pm$ 11.78	150.50 $\pm$ 9.72	133.67 $\pm$ 10.67
Group III	86.83 $\pm$ 2.45 @***	223 $\pm$ 19.88 @***	229 $\pm$ 14.93 @***	314.83 $\pm$ 18.31 @***
Group IV	85.33 $\pm$ 1.75 @***	221.67 $\pm$ 14.9 @** \$***	180.17 $\pm$ 6.31 @*** \$***	167.67 $\pm$ 8.27
Group V	87 $\pm$ 1.83 @***	230.67 $\pm$ 20.69 @** \$***	175.50 $\pm$ 5.49 @** \$***	150.83 $\pm$ 8.73
Group VI	84.17 $\pm$ 3.28 @***	227.17 $\pm$ 21.78 @* \$***	169 $\pm$ 10.72 @* \$***	140.17 $\pm$ 5.10

Table 3 shows blood glucose level weight of various groups in 14<sup>th</sup>, 19<sup>th</sup>, 28<sup>th</sup> and 42<sup>nd</sup> days of the experiment, and values are denoted as Mean  $\pm$  SEM (n = 6 animals each) and significance at \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ . @ - compared with control; \$ - compared with streptozotocin-induced diabetic animals (Group III).

### Blood Glucose Level @\*\*\*



**Graph 3** demonstrates increased blood glucose level in group III after the induction of streptozotocin. Significance at \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ . @ - comparison with control.

### Histological study

The histological study of the Islet of Langerhans by Harris hematoxylin and Eosin has been shown in fig.1. Group I shows the normal architecture of the islets of Langerhans with round to oval shape a typical arrangement of acinar cells that appear in a radial orientation around the lumen.

In group II, high fat diet animals showed the presence of shrunken or relatively large islets of Langerhans. In group III, diabetic induced animals showed degenerative changes a little more severe that specified swollen acinar cells, shrunken or deformed shape of islets, and infiltration of inflammatory cells in the pancreatic islets. The cells in the islets, particularly beta cells, have either deformed nuclei or lost entirely, whereas in the animals treated with *Gymnema sylvestre* in both low and high dosages (Group IV and Group V), the degenerative changes were minimized, showing normal arrangement of serous acini. These two groups showed regeneration of islets of Langerhans and an increased number of beta-cells. This recovery is close to the rats in Group VI. A similar observation was reported after supplementation of *Gymnema sylvestre* in diabetic rats (Hafizur, 2015).

**Fig. 3 Histology of pancreas**

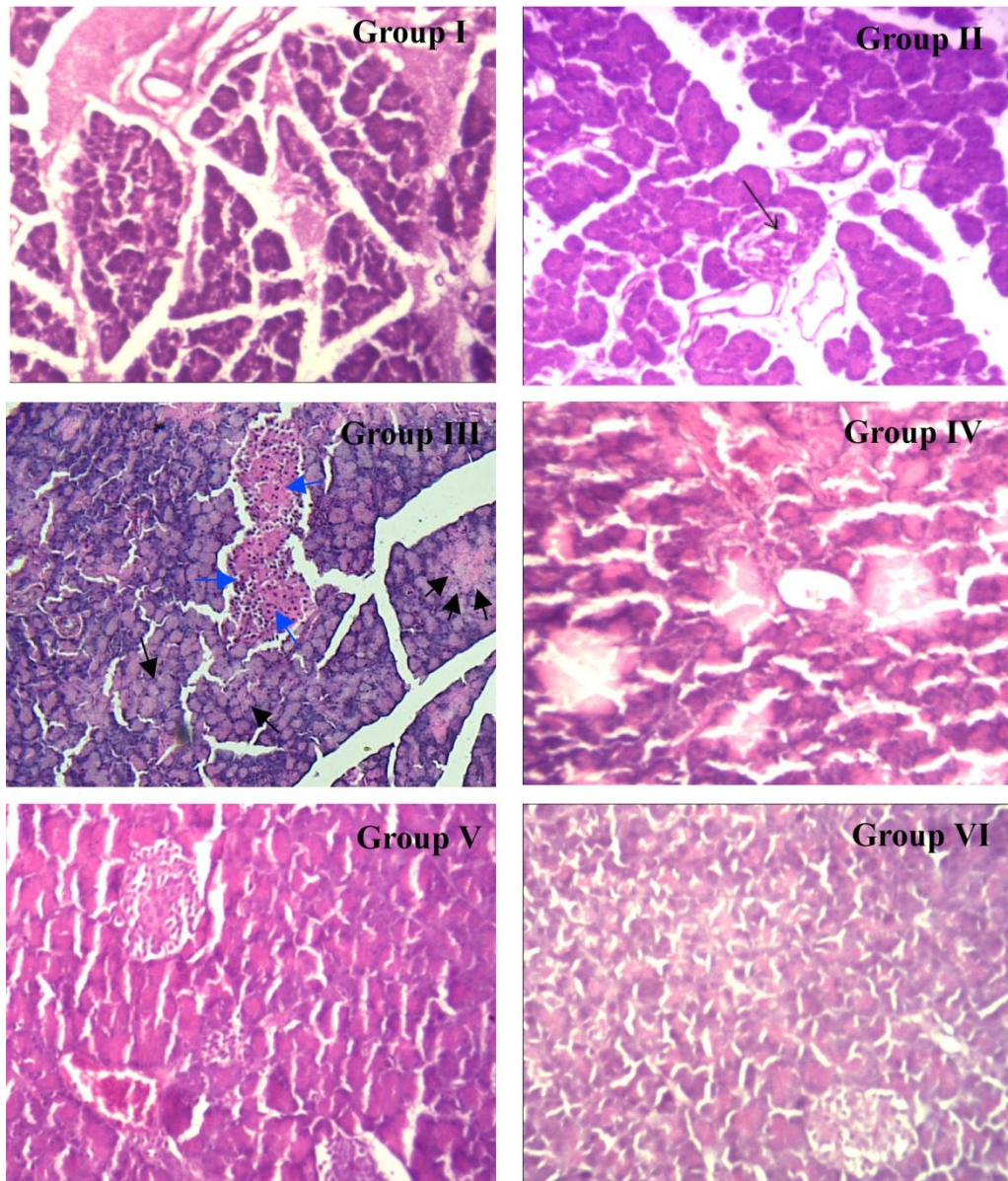


Fig.3 shows the histology of the pancreas of different experimental animals. Group I showing normal architecture of the pancreas. Group II showing shrunken islets of pancreas. Blue arrows indicating shrinkage of Islets of Langerhans and black arrows are pointing damage of acini in the diabetic animal. Group IV, V and VI are treatment groups showing recovery of normal architecture of the islets of Langerhans

#### Immunohistochemistry Fig. 4

The immunohistochemistry of GLUT-2 protein [Fig.4] in the pancreas appeared as a darkly stained area with brown (arrow mark). When compared to the control (Group I), GLUT-2 expression was significantly lower ( $P < 0.001$ ) in high-fat diet and streptozotocin-induced diabetic animals (i.e., Group II and Group III). Conversely, the pancreas of *Gymnema sylvestre* and metformin-treated animals (Group IV, Group V, and Group VI) indicated the up-regulation of GLUT-2 activity ( $P < 0.001$ ) when compared to diabetic animals (Group III). Although GLUT-2 expression was increased in group IV with a low dose of *Gymnema sylvestre* administration, the improvement was remarkable at a high dose [Graph 4]. These changes in GLUT-2 expressions were measured using Image analysis J 1.46 software to substantiate immunohistochemistry of GLUT activity [Table 4].

**Table 4 Relative Expression of GLUT-2**

Groups	Relative Expression of GLUT-2
Group I	$6.58 \pm 0.121$
Group II	$3.56 \pm 0.103$ @***
Group III	$2.24 \pm 0.096$ @***
Group IV	$4.51 \pm 0.137$ @* \$***
Group V	$5.16 \pm 0.172$ \$***
Group VI	$5.68 \pm 0.195$ \$***

**Graph.4**

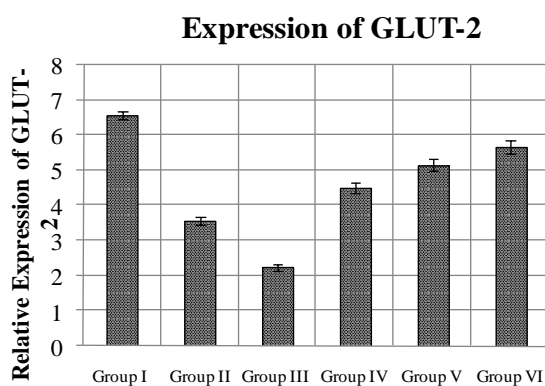


Table 4 and Graph 4 showing the Relative Expression of GLUT-2, quantified by the Image analysis J 1.46 software and the values represent Mean  $\pm$  SEM with significance at \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . @ - comparison with control; \$ - comparison with streptozotocin-induced diabetic animals.

**Fig. 4 Immunohistochemistry of GLUT-2**

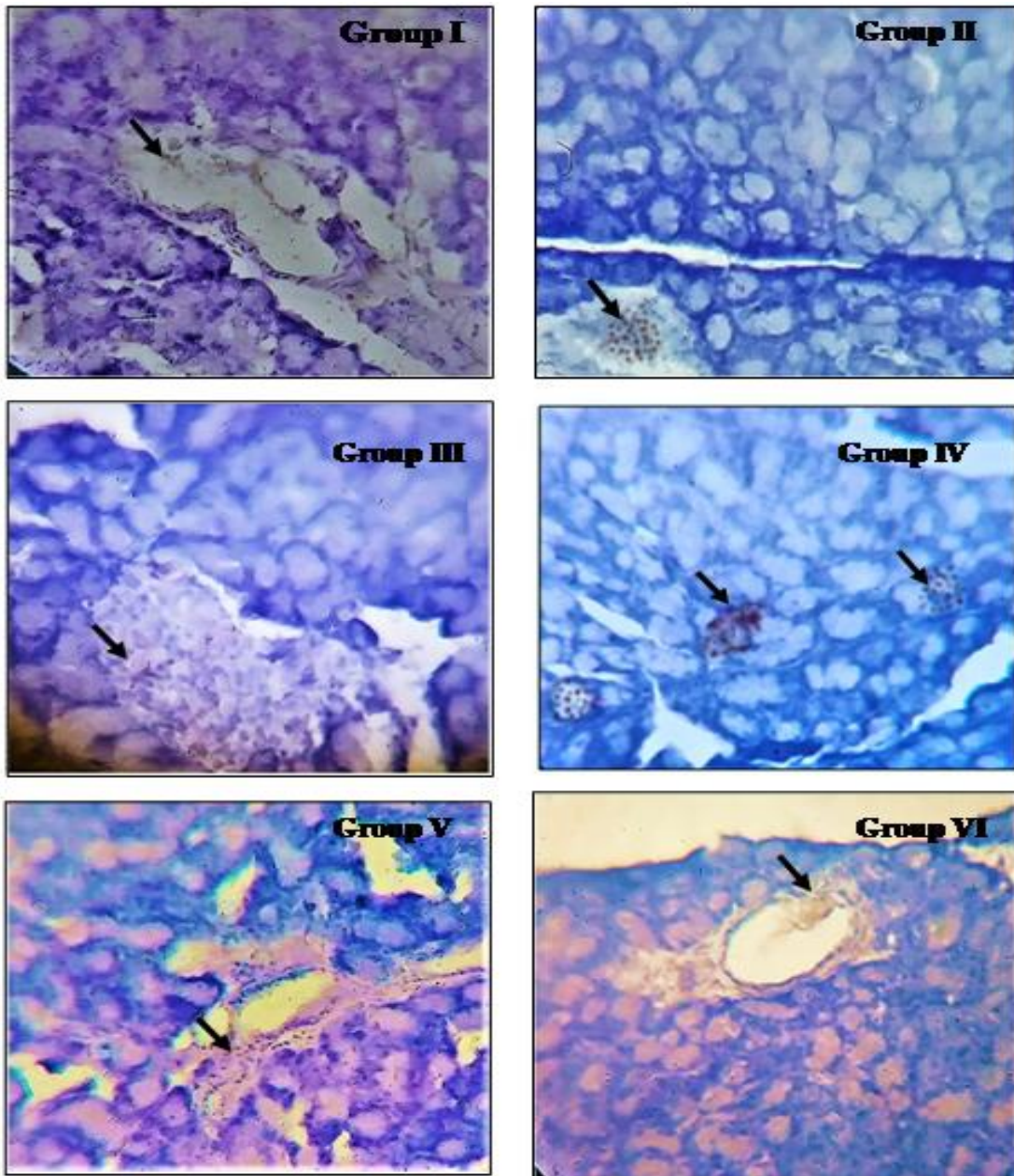


Fig.4 demonstrates immunohistochemistry of GLUT-2 in the pancreas of control and various experimental animals. Arrows indicating the darkly stained area with brown shows the expression of GLUT-2.

## Discussion

In the present study, the methanolic extract of *Gymnema sylvestre* was administered to diabetic rats and the effectiveness of the same was evaluated in comparison to metformin. The effect of *Gymnema sylvestre* was almost similar to that of metformin, a fundamental drug for diabetes and the possibility of replacing it by herbal preparation of *G. sylvestre* for diabetes treatment.

On agreement of our present results a recent study demonstrated the protective effects of *Gymnema sylvestre* against type 2 diabetes mellitus and its associated abnormalities (Dhananjay Yadav et al., 2019). The experiment in diabetic rodent model treated with *Gymnema sylvestre* suggested that the anti-diabetic effect could be achieved by the improvement in glycogen synthesis, glycolysis, gluconeogenesis, and hepatic and muscle glucose uptake (Porchezian and Dobriyal 2003) and facilitated the hinder of hemoglobin and protein glycosylation (Shanmugasundaram ERB et al., 1988). Kumar et.al., 2014, reveal that water soluble fraction of *Gymnema sylvestre* ethanol extract could be useful intervention in the treatment of obesity and type-2 diabetes mellitus. The ameliorating effects of the *Gymnema sylvestre* could be attributed to the bioactive components present in this herbal drug chiefly the saponin. The gymnemosides and gymnemic acid are saponin components responsible for the antihyperglycemic effect of *Gymnema sylvestre* (Murakami et al., 1996).

### Animal Body weight & Organ Weight

We observed that streptozotocin-induced diabetes results in decreased body weight i.e., group III which is according to the Al-Shamaony et al. (1994). This weight loss might be due to loss of body muscles of rats with diabetes (Swanston-Flatt et al. 1990). A single intraperitoneal or intravenous injection of STZ is well reported to induce insulin-dependent diabetes in rats (Ghorbani et al., 2010; Ghorbani et al., 2013). Consistent with our results, treatment of diabetic rats with gymnema also improved the body weight when compared with untreated diabetic rats, this result is confirmed by Okokon et al., 2012. Here, we also showed that body weight of the diabetic (untreated) rats was reduced significantly in comparing with to the control group.

The decrease in the pancreas weight in streptozotocin-induced diabetic animals could be the result of disruption of pancreatic islets and selective destruction of insulin-producing cells (Kim et al., 2006; Heidari et al., 2008; Zafar et al., 2010). The administration of *Gymnema sylvestre* (both low and high dosage) were noticeably maintained the animal body weight as well as weight of the pancreas towards the normal undoubtedly which indicates the effects of the herbal drug. These results were further correlated with the known drug metformin-treated diabetic animals. But in case of liver, we didn't come across any significant reduction in their weight.

### **Blood glucose levels**

A study stated that *Gymnema sylvestre* reduced the fasting glucose levels together with a considerable lowering of serum lipid levels and concomitantly ameliorating serum protein levels (Shanmugasundaram KR et al., 1981) substantiated protective effect of this herbal drug in diabetic animals. In vitro studies shown that the alcoholic extract of *Gymnema sylvestre* in HIT-T15, MIN-6, and RINm5F  $\beta$ -cells stimulates the release of insulin by increasing membrane permeability (Persaud SJ, et al., 1999; Sheoran et al., 2015) through a channel-independent influx of  $Ca^{++}$  into the  $\beta$ -cells thereby maintains blood glucose level. Thus, in our study there is a significant increase in blood glucose levels in the high dose of *Gymnema sylvestre*. However both dosage groups were restoring blood glucose level proved to be the protective effect of *Gymnema sylvestre* administration.

### **Histology**

Islets of Langerhans of diabetic animal shows loss of shape and presence of vacuolated and a few necrotic cells. Proliferation of fibroblasts with elongated nucleus (Mallikarjuna et.al., 2013) . The gymnemic acids of *Gymnema sylvestre* have been reported to have direct action on pancreatic  $\beta$ -cells and may bring about the regeneration or repair of beta cells leading to increase in serum insulin levels (Shanmugasundaram et al., 1990; Persaud et al., 1999 and Kanetkar et al., 2007). This was also supported by the histopathological examination of the present study.

Insulin is a main hormone secreted by the beta cells of islets of Langerhans. Streptozotocin causes selective destruction of beta cells, supporting our histopathology analysis (Ahmed et al., 2000). The gymnemic acids of *Gymnema sylvestre* have been reported to have direct action on pancreatic  $\beta$ -cells and may bring about the regeneration or repair of beta cells leading to increase in serum insulin levels (Kanetkar et al., 2007). This was also supported by the histopathological

examination of the present study. Gross pathological changes observed in the present group reduced progressively with advancement in time on treatment with metformin. The results were in agreement with our findings (Ananthan et al. 2003).

The progressive improvement in the microscopic pathology of islets of Langerhans attributed to increased proliferation as well as recruitment of subpopulation of beta cells and thereby increases in the beta cell mass upon treatment with glibenclamide (Guiot et al., 1994 and Wang et al., 2008). This result corresponds with our study in the histopathological observations of islets of Langerhans. In the present study the hypoglycemic effect of *Gymnema sylvestre* was almost similar to that of metformin, a cardinal drug for diabetes and provokes the possibility of replacing it by herbal preparation of *G. sylvestre* for diabetes treatment.

### **Immunohistochemistry**

The GLUT is the facilitated diffusion glucose transporters expressed in the plasma membrane of insulin-positive beta cells and required for glucose-stimulated insulin secretion (Benard 2015) and it is the major glucose transported in islets of beta cells (Piero marchetti et al., 2017; Rosa gasa 2016;Becca 2017). The down regulation of GLUT-2 expression in high-fat diet and type 2 diabetic animals' demonstrated impairment of beta-cell function. The study suggested that the risk of type 2 diabetes and obesity assumed to have the primary defect in insulin action probably by a single nucleotide polymorphism in genes that regulates insulin secretion (Olli Laukkanen et al., 2005). Another earlier study further supports our GLUT-2 results stated that the loss of GLUT-2 in pancreatic cells is an early indicator of beta-cell dysfunction (Bonny et al., (1997) commencing diabetes. The treatment of *Gymnema sylvestre* at both dosages and metformin restored the GLUT 2 expression in diabetic induced animals. Interestingly high dosage of *Gymnema sylvestre* administration showed remarkable improvement. The findings revealed that *Gymnema sylvestre* enhance the expression of GLUT-2 dose-dependently. Ahmed et al, (2010) suggested *Gymnema sylvestre* exerts antidiabetic activities through the regeneration of beta cells in alloxan-induced diabetic rats. Likewise other studies proved a cluster of beta-cell regeneration in the diabetic rat (Hafizur 2015) after the treatment of *Gymnema sylvestre*. Our histological of pancreatic tissue also confirm the regeneration of beta cells. Supporting our GLUT-2 result, the methanolic leaf extract of *Gymnema sylvestre* has shown increased glucose uptake and facilitated the enhanced GLUT-4, an isoform of glucose transporters (Mahesh Kumar et al., 2016). Regenerative effect of *G.*

sylvestre has been also observed following long-term treatment of diabetic rats using a standardized dry extract of leaves (Aralelimath, Bhise, 2012). Gymnemic acid is considered to be the active ingredient responsible for the regenerative action of *G. sylvestre* on beta cells (Ahmed, Rao, Rao, 2010)

In our immunohistological study, the appearance of dark stain brown areas in slide indicates the presence of GLUT-2 which is prominent in the treatment group of high dose of *Gymnema sylvestre*. This fact enumerates the formation of GLUT-2 can occur due to the management of ethanolic extract of *Gymnema sylvestre*. The same mechanism could be orchestrated in the expression of GLUT-2. Thus it could be suggested that the effectiveness of *Gymnema sylvestre* could be attributed to the augmentation of glucose transporters and glucose absorption. In agreement with earlier reports our study suggest that *Gymnema sylvestre* regenerate and/or restore the pancreatic beta cells via increasing glucose uptake enabled the improved GLUT-2 expression. However the possible link between the antidiabetic activity of *Gymnema sylvestre* and GLUT-2 expressions remain to be explored.

On the whole of our study we suggested that the *Gymnema sylvestre* could be a potential herbal drug that prevents the deteriorating effect of diabetes via the regeneration of  $\beta$ -cells and regularized blood glucose level.

## **Conclusion**

In the current investigation, we concluded that the *Gymnema sylvestre* treatment improved the body weight, maintain pancreas weight and blood glucose level, the high dosage of *Gymnema sylvestre* were more effective. The *Gymnema sylvestre* protects the pancreatic beta cells to sustain glucose transporter GLUT-2 level and the bioactive components, saponin present in this herbal drug could be responsible for its ameliorating effects. Thus *Gymnema sylvestre* could be a potential herbal drug, would be a better choice for the treatment of type2 diabetes. However further studies are required before clinical implication.

## **Ethical Approval**

The present study was conducted according to a protocol approved by the animal ethical committee of the Sathyabama Institute of Science and Technology (No. SU/CLATR/IAEC/XI/100/2018) and the CPCSEA (2003) guidelines.

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

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