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EFFECT OF AN ISOLATE FROM GYMNEMA SYLVESTRE, R. BR. IN THE CONTROL OF DIABETES MELLITUS AND THE ASSOCIATED PATHOLOGICAL CHANGES

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ABSTRACT: *Gymnema sylvestre, R. Br., popularly known as Meshashringi in Sanskrit and Sarkaraikolli in Tamil, was investigated for the control of type I (insulin dependent) diabetes in experimental animals. The hypoglycaemic extract was found to bring about blood glucose homeostasis, by increasing serum insulin levels. The islets of langerhans appear to be restored or regulated by the herbal extract. Increased glycoprotein, which is the major metabolic abnormality in diabetes mellitus and the resultant nephropathy, retinopathy and micro and macroangiopathy, is brought under control by the administration of the leaf extract.*

INTRODUCTION

Sushruta (6^{th} century B.C.a) and C' araka (1^{st} century A. D), teachers and documentralists of Ayurveda have recommended the use of the leaves of the 'Sala Saradi' group for the control of maturity onset diabetes in addition to diet restriction. *Gymnema sylvestre,* R. Br. is a plant belonging to this group and has been described in "Indian Medicinal Plants" (Blater *et al,* 1930). The medicinal properties of its roots and leaves have been described in the 'Indian Materia Medica' (Nadkarni, 1954). Sushruta (6^{th} century B.C.b) described this as destroyer of 'madhumeha' (glycosuria) and other urinary disorders.

The modern scientific investigations of the medicinal effect of the leaves of *Gymnema sylvestre* was initiated by Mhaskar and Caius (1930). They found phytol, myoinositol and scyllitol as components of the leaves of *G. sylvestre.*

Gymnema sylvestre is a woody branched climber commonly found growing wild in unkempt forests of Central India and the Western ghats. The plant is known by different names in different languages. It is known as *Periploca of the woods* in English; *Waldschilinge* in German; *Meshashringi* and *Sarpadarushtrika* in Sanskrit; *Gurmar* in Hindi; *Kogilam, Shirukurinja* and *Sarkarikkolli* in Tamil and *Podapatri* in Telugu (Sastri, 1956).

Edgeworth first noticed the sweetness abolishing property (Antisaccharine property) of *G. sylvestre* leaves and this was confirmed by Hooper (1887) who isolated gymnemic acid (Hooper, 1889). Power and Tutin (1904) reported the presence of gymnemic acid, as glucoside with anti-saccharine property and hentriacontane in *G. sylvestre* leaves. Gharpurey (1926) discovered that the leaves of *G. sylvestre*

given to diabetics reduced urine sugar levels.

In our laboratory, various aspects of glucose metabolism in diabetes and its control by *Gymnema sylvestre* have been studied. Panneerselvan and Shanmugasundaram (1978) reported the hypoglycaemic activity of the leaf powder in normal and diabetic men and rabbits. They reported that *G. sylvestre* administration is accompanied by 15 to 30 per cent reduction in the blood sugar level in the fasting state and postprandial condition both in normal and moderate diabetics in humans. The extraction of the hypoglycaemic component of the leaves (GS_2) and use of it in the control of blood glucose levels in diabetes induced by alloxan was studied by Shanmugasundaram *et al* (1981).

The acid precipitate of the alcoholic extract (GS_3) was used in further investigations. Rajendran and Shanmugasundaram (1980) have shown that the intestinal uptake of glucose, amino acids and water increases during the diabetic state and this alteration is reversed upon GS_3 administration. The abnormal functional activity of the intestinal brush border membrane during diabetes was corrected upon GS_3 administration (Rajendran, 1981).

The investigations presented in this paper involved the use of a further product of the herb, GS_4 in correcting the metabolic abnormalities observed on inducing diabetes by streptozotocin injection to rats.

MATERIALS AND METHODS

Albino rats of both sexes used for the experiments were obtained from the inbred stock derived from Wistar stock of animals from the King Institute of Preventive Medicine, Madras. They were maintained on Hindustan Lever rat feed and given water *ad libitum.* The chemicals used in this investigation were obtained as follows.

Standard bovine serum albumin, crystalline insulin from M/s. Sigma Chemicals St. Louis, U. S. A., acetylacetone, orcinol, sodium metaperiodate from Glaxo Chemicals (BDH), Poole, England, p-dimethylaminobenzaldehyde, galactose, glucosamine hydrochloride and sodium arsenite from E. Merck, Dermstadt, West Germany; N-acetylneuramnic acid from Boehringar Mannheim, West Germany and insulin 'kit' from Bhabha Atomic Research Centre, Bombay, India.

The plant material was collected from Marimrakula Kandriga in Chittoor District of Andhra Pradesh. The plant which is a climber grow well in the bushy forests on the hills in this area. The leaves are air dried in the shade, extracted with aqueous ethanol (50/50, v/v) and precipitated with HCl. The precipirate which showed hypoglycaemic activity was purified by recrystallisation from aqueous alcohol. This was labeled GS_4 and used for controlling blood glucose levels.

Induction of diabetes mellitus and the experimental design

Adult albino rats (weighing between 150 and 160 g) of both sexes were fasted overnight and streptozotocin dissolved in 0.9 per cent NaCl at pH 4.5 was administered intracardially at a dosage of 55 mg/kg body weight. Animals with a fasting blood sugar value between 150-200 mg/dl were termed as diabetic.

For investigation, the experimental animals were divided into 3 groups. Group I had a single injection of saline at the beginning of the experiment. A group of rats were injected with streptozotocin and were

maintained hyperglycemic for the first four weeks by providing food and water *ad libitum.* At the end of four weeks the animals of group III were administered the drug GS_4 at a dose of 20 mg per day per rat, mixed with powdered and moistened feed. Each rat was fed separately and continued with food and water *ad libitum.* After 8 weeks of treatment the drug was discontinued to group III animals and the animals of all the three groups were maintained a further period of 4 weeks with food and water *ad libitum.* At the end of 16 weeks (4 weeks after the drug was discontinued in group III), glucose tolerance test was performed and the animals of all the groups were sacrificed for analyses.

Liver, kidney, skeletal muscle, epididymal fat and intestine were removed quickly into ice-cold containers. Blood was collected in tubes containing EDTA as anticoagulant and plasma was separated. The red blood cells were washed with 0.9 per cent NaCl and mixed with an equal volume of distilled water to give a hemolysate.

Blood sugar and serum insulin levels were determined in the blood collected during the glucose tolerance test (GTT) by the method of Folin and Wu (1920) and radiommunoassay procedure of Herbert *et al* (1965) respectively. Glycosylated haemoglobin was estimated by the method of Wang and Yang (1982) in the hemolysate. Glycosylated plasma protein was estimated by the method of Merelyn *et al* (1981).

While extracting the lipids from tissue, the residual protein left out was hydrolysed for the carbohydrate components bound to protein according to the method of Tettamanti *et al* (1973). The components analysed in the hydrolsate were hexose (Niebes, 1972), hexosamine (Wagner, 1973), and sialic acid (Warren, 1959).

For statistical analysis, students't' test was performed and 'p' values arrived at. The untreated diabetic animals were compared to the normal, while those under GS_4 therapy were compared with the untreated diabetic rats of group II. Pearson's correlation coefficient was obtained according to the method of Snedecor and Cochran (1967) and the glycosylated protein was correlated with blood glucose level.

RESULTS

Figure I show the control of blood glucose by GS_4 therapy. The glucose levels of diabetic rats of group II is deteriorating with time, both the fasting and the post prandial levels being higher than that observed 4 weeks before. The animals of group II (on GS_4 therapy) show improvement in glucose tolerance at 8^{th} week and progressing in the 12^{th} week. The glucose tolerance curve remains the same 4 weeks after the withdrawal of GS_4 therapy.

Figure II shows the insulin levels measured in serum obtained from the blood collected during GTT. The levels are considerably lowered in the diabetic animals, and the rise in insulin levels is poor in hyperglycaemic condition. In the animals after GS_4 therapy even after the discontinuation of the drug, the insulin levels in both the fasting and post-prandial conditions are higher indicating that the β-cells have regenerated.

Figure III depicts the correlation between fasting blood glucose and glycosylated haemoglobin levels. A significant positive correlation is found among the control, diabetic and drug treated animals between these two parameters whose coefficients of

correlation are 0.89, 0.99 and 0.95 respectively in the three groups.

The correlation between the fasting blood glucose and glycosylated plasma proteins is depicted in Figure IV. As in the case of glycosylated haemoglobin, a significant positive correlation is also found between fasting blood glucose and glycosylated plasma protein in the normal and experimental animals.

The pattern of changes in the protein bound carbohydrates components in the liver, kidney, adipose tissue skeletal muscle and intestine can be seen in Table I. In the diabetic rat liver and kidney, protein-bound hexoses are increased significantly. Hexosamines bound to protein are also elevated. Sialic acid bound to protein is increased in the liver while it is lowered in the kidney. There is near normalization by GS_4 of these components. In the adipose tissue, while both hexose and sialic acid are elevated, the increase observed for sialic acid is more significant. However, hexosamine shows a reduction in the diabetic group which is reversed in the controlled diabetic state (Group III). Hexose and sialic acid register a moderate, but statistically significant increase in the diabetic skeletal muscle which is completely normalized by GS_4 therapy. In the intestine, protein- bound sialic acid and hexosamine are lowered in the diabetic state, while hexoses are increased. The reversal by GS_4 therapy is not fully complete.

These observations indicate that GS_4 administration to diabetic animals brings about blood glucose homeostasis and also brings back the glycoconjugate components to near normal levels.

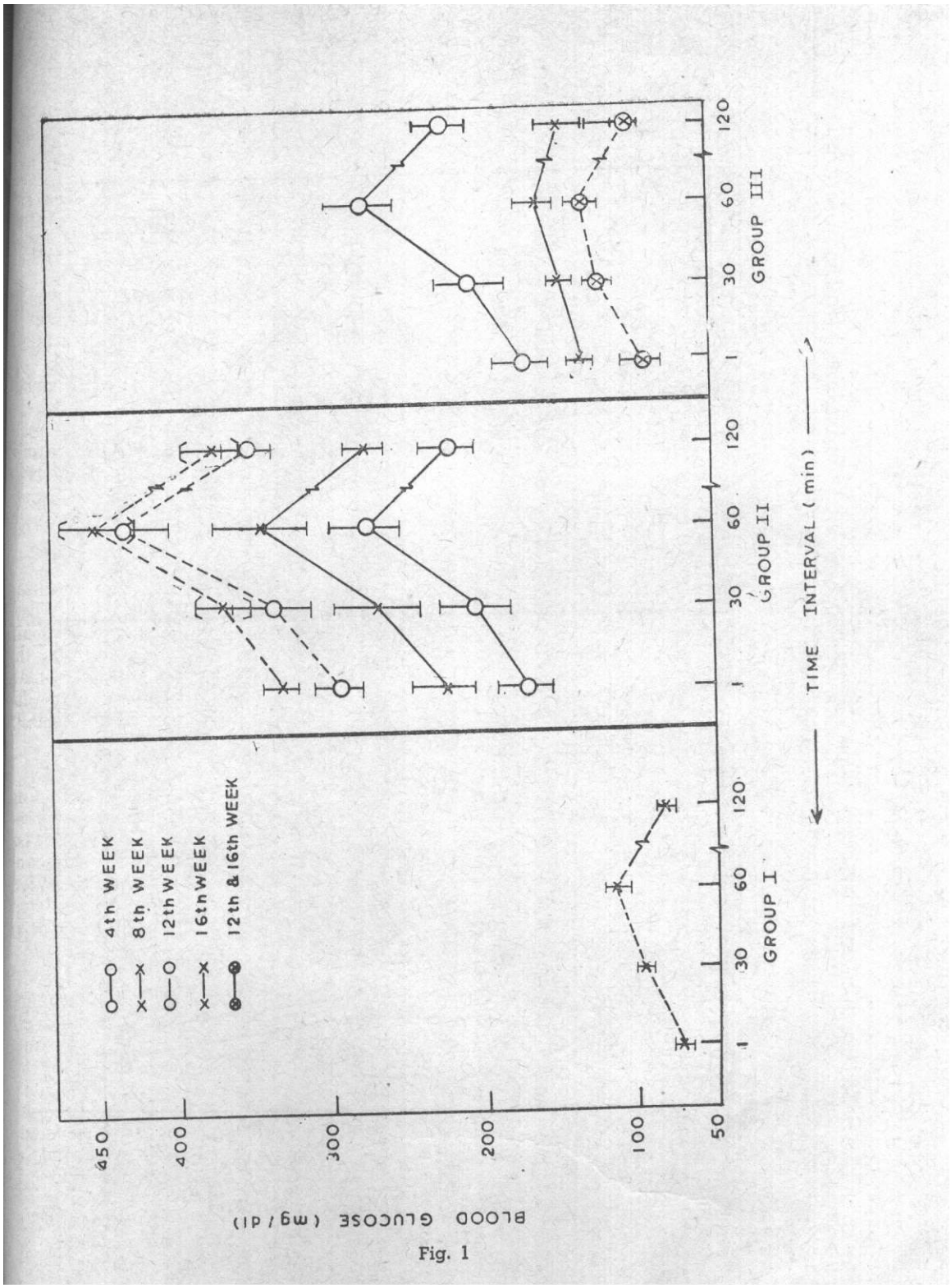
BLOOD GLUCOSE (mg/dl)
450
400
300
200
100
50
4th WEEK
8th WEEK
12th WEEK
16th WEEK
12th & 16th WEEK
GROUP I
GROUP II
GROUP III
1
30
60
120
TIME INTERVAL (min)

Fig. 1

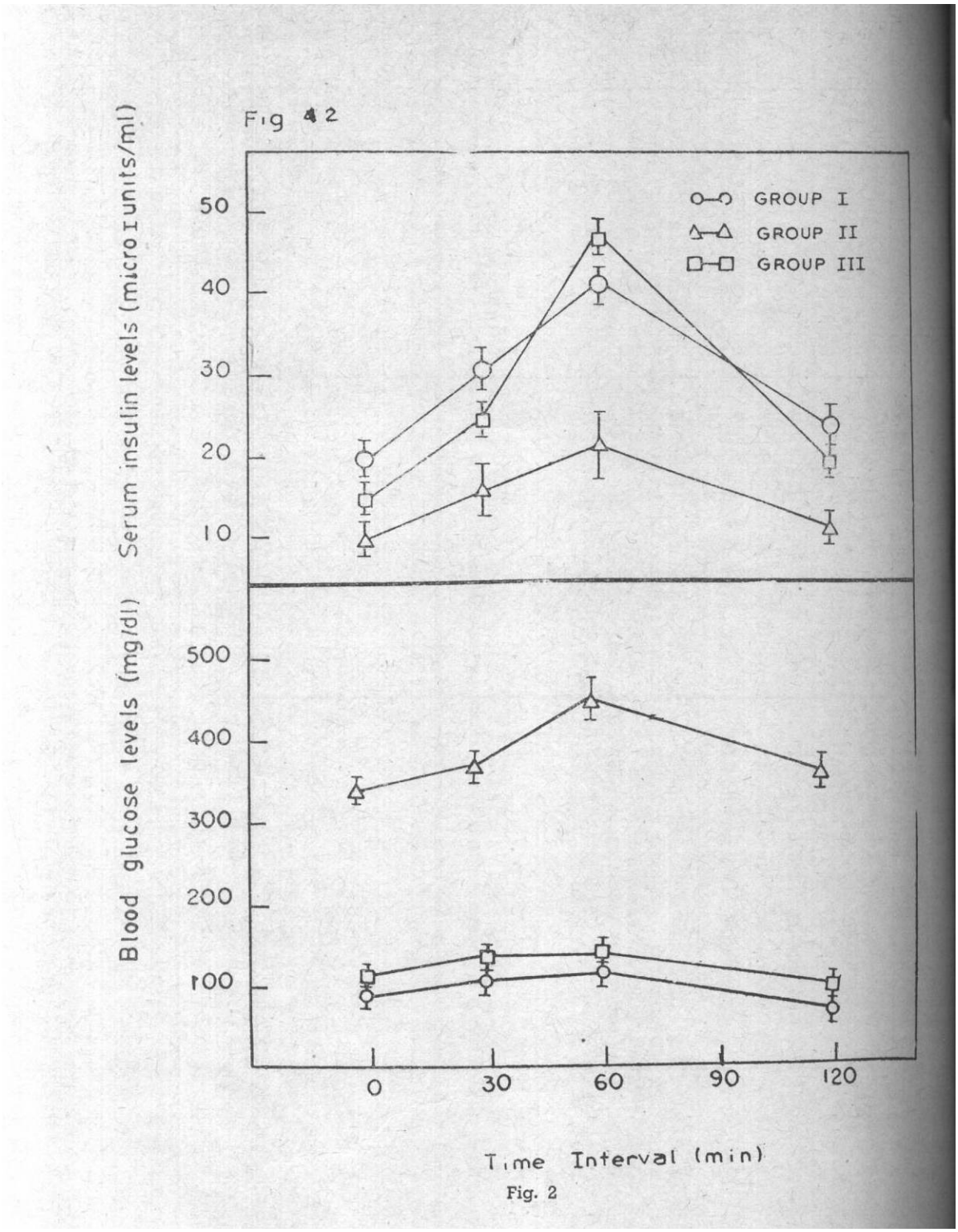
Fig 42
GROUP I
GROUP II
GROUP III
Serum insulin levels (micro units/ml)
50
40
30
20
10
Blood glucose levels (mg/dl)
500
400
300
200
100
0
30
60
90
120
Time Interval (min)

Fig. 2

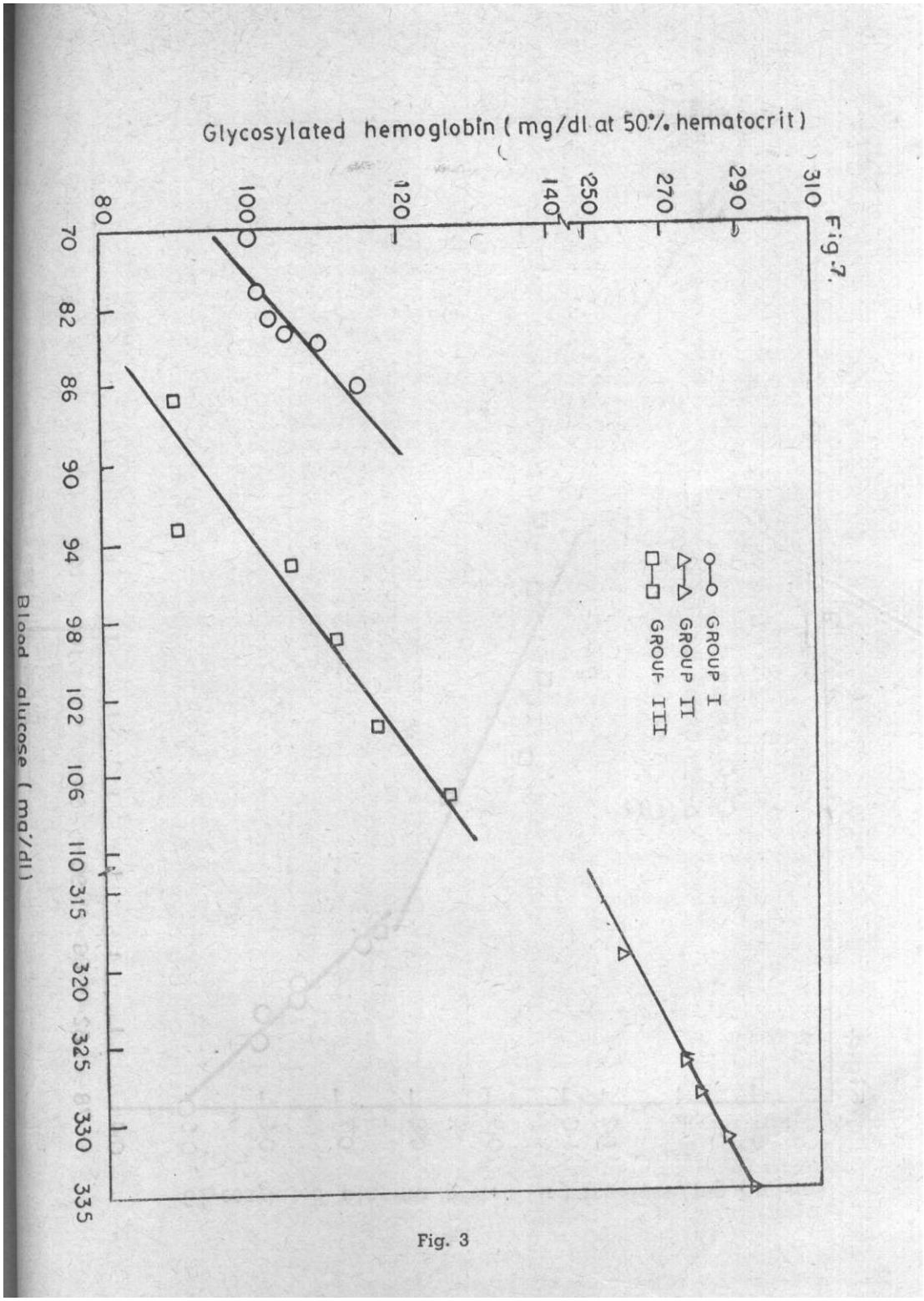
Glycosylated hemoglobin (mg/dl at 50% hematocrit)
Fig.7.
80
100
120
140
250
270
290
310
70
82
86
90
94
98
102
106
110
315
320
325
330
335
GROUP I
GROUP II
GROUP III
Blood glucose (mg/dl)

Fig. 3

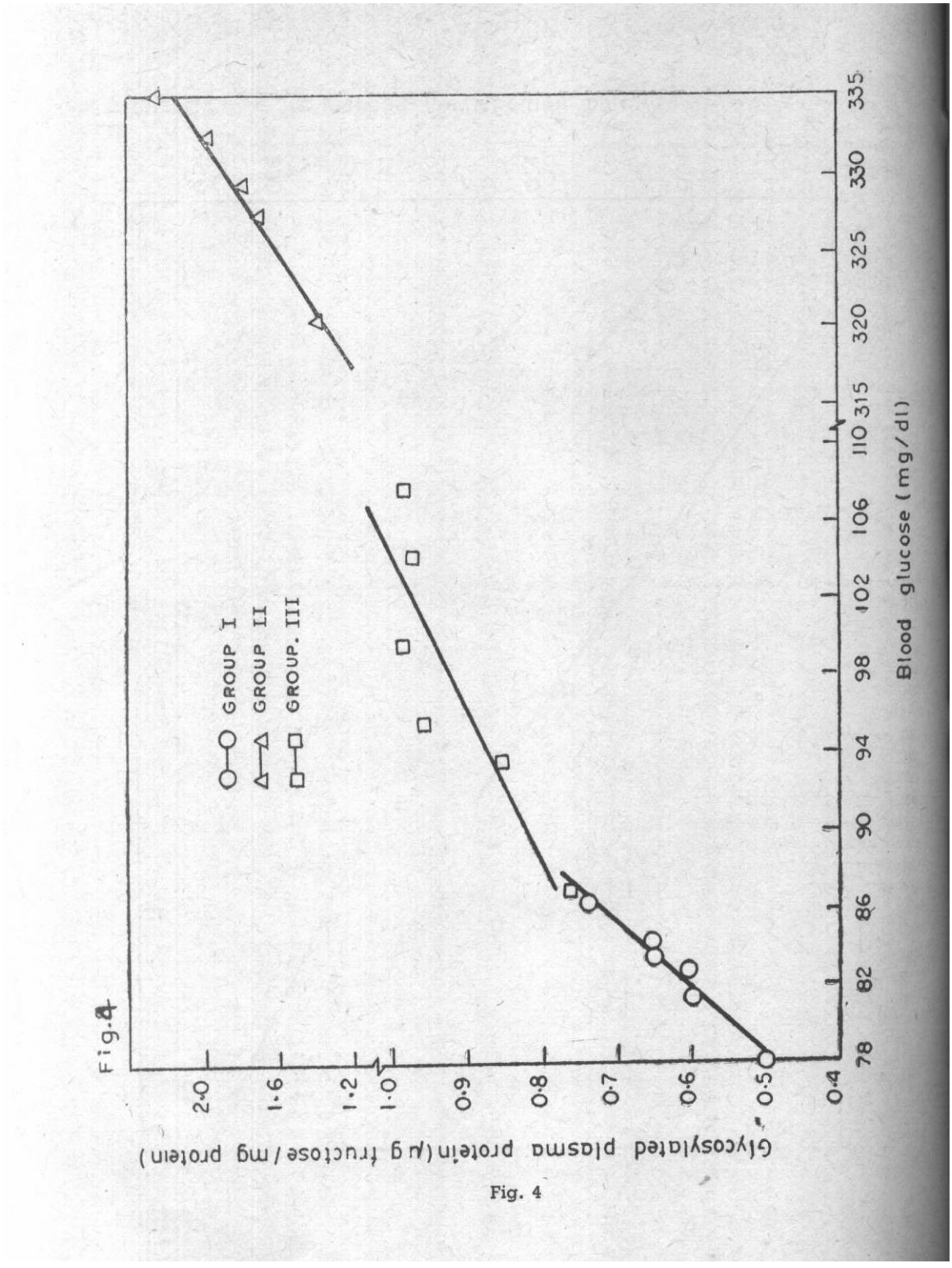
Fig.4
GROUP I
GROUP II
GROUP. III
Glycosylated plasma protein (μg fructose / mg protein)
Blood glucose (mg/dl)
2·0
1·6
1·2
1·0
0·9
0·8
0·7
0·6
0·5
0·4
78
82
86
90
94
98
102
106
110 315
320
325
330
335

Fig. 4

TABLE – I

Protein – bound carbohydrate components in liver, kidney, adipose tissue, skeletal muscle and intestine of control and experimental animals at the end of 16 weeks. Values are expressed as mg/g of defatted tissues and are mean ± S. D. for 'n' number of animals.

Tissue		Group I (n = 6)	Group II (n = 6)	Group III (n = 6)
Liver	Hexose Hexosamine Sialic acid	19.09 ± 2.91 20.04 ± 2.20 5.58 ± 0.79	39.31 ± 2.70 *** 31.02 ± 2.00 *** 10.43 ± 1.60 ***	22.00 ± 1.70 *** 20.35 ± 2.12 *** 5.75 ± 1. 51 **
Kidney	Hexose Hexosamine Sialic acid	44.16 ± 5.80 21.24 ± 3.61 9.77 ± 2.04	53.49 ± 4.73 * 33.56 ± 4.50 *** 5.63 ± 0.81 **	42.63 ± 3.23 ** 22.76 ± 2.52 *** 7.19 ± 1.61
Adipose tissue	Hexose Hexosamine Sialic acid	54.65 ± 6.50 7.86 ± 1.06 9.29 ± 0.73	64.81 ± 7.20 * 6.20 ± 0.51 * 13.77 ± 0.57 ***	57.32 ± 4.80 7.41 ± 0.73 * 11.45 ± 0.63 ***
Skeletal muscle	Hexose Hexosamine Sialic acid	25.20 ± 2.70 13.33 ± 1.13 2.39 ± 0.25	34.74 ± 2.69 *** 8.80 ± 1.16 *** 3.47 ± 0.25 ***	23.40 ± 3.35 ** 12.09 ± 1.37 ** 2.49 ± 0.33 **
Intestine	Hexose Hexosamine Sialic acid	25.90 ± 2.70 13.12 ± 0.45 13.90 ± 1.60	34.60 ± 3.20 ** 7.84 ± 0.85 *** 7.47 ± 0.97 ***	27.30 ± 1.40 ** 10.79 ± 1.06 ** 10.40 ± 1.30 **

For statistical significance group II was compared with group I and group III was compared with group II

Significant variations are indicated as : * p 0.05; ** p 0.01; *** p 0.001

DISCUSSION

From the observations reported in Figure I it can be seen that the hypoglycaemic extract of *Gymnema sylvestre*, R. Br., labeled GS_4 is able to bring back blood glucose homeostasis. It is also interesting to note that 4 weeks after the drug was discontinued (from 13^{th} to 16^{th} week) the blood glucose levels during GTT remained at the same level as at the conclusion of drug therapy in 12^{th} week. It is also noted that this regulation of blood glucose levels is associated with increase in both glucose levels is associated with increase in both fasting and post-prandial serum insulin levels as a response to a glucose load (Fig. II). The lower serum insulin levels found in the diabetic animals even after a glucose load (post-prandial condition) is corrected by the drug as seen by the increase in the serum insulin levels of group III animals. GS_4, therefore facilitates the insulin release in response to glucose load. Thus, GS_4 makes available circulating insulin levels which will correct metabolic abnormalities in diabetes. Streptozotocin induced diabetes is a classical model of the type I or insulin dependent (juvenile) diabetes, in which the bulk of the insulin producing β-cells of the islets of Langerhans are destroyed. Hence it is observed that the serum insulin in the fasting is low and that the response of the pancreas to increased blood glucose level is only marginal with the result that increase in serum insulin levels after glucose ingestion is insufficient. However, after GS_4 therapy both fasting and post-prandial serum insulin levels are elevated indicating that the number of functional β-cells in the pancreatic islets of Langerhans has increased by therapy. This may suggest a regeneration of β-cells. Histopathological studies (Fig. Va to Vc) reveals that the islets of Langerhans are intact after therapy, confirming the earlier findings.

Glycosylated haemoglobin levels (Fig. III) are correlated to the blood glucose levels. Similar correlations are observed between the glycosylated plasma protein levels and fasting blood glucose presented in Figure IV. The glycosylation of both haemoglobin and plasma proteins are significantly greater in the diabetics and this is reversed by GS_4 therapy.

Glycosylation of haemoglobin interferes in the erythrocyte function. In diabetics, where a relative deficiency of 2, 3 – diphosphoglycerate (2, 3-DPG from glycolysis) may co-exist with elevated glycohaemoglobin levels during periods of changing blood glucose concentration, may result in decreased oxygen delivery to critical tissues (Standl and Kolb, 1973).

Casparie and Miedema (1977) have also reported such changes. Davis and Nicol (1980) reported that insulin treated diabetics with retinopathy had a raised level of glycosylated haemoglobin compared with those without this complication, but no such association was found in those controlled with oral hypoglycaemics. Treatment of diabetic rats with GS_4 resulted in bringing back the raised glycosylated haemoglobin levels, indicating the superiority of GS_4 over insulin.

From Table 1, it can be seen there is a pattern of changes in the protein-bound carbohydrate components. Increased protein deposition of hexose and hexosamine is observed in the liver and kidney of diabetic rats. While sialic acid content increased in the liver it decreased in the kidney of diabetic rats. After GS_4 treatment the levels are normalized. In the adipose tissue and skeletal muscle, protein-bound sialic acid

levels are low, and they show a mild increase in diabetes. The intestinal sialic acid is decreased when blood glucose is under control as in group III. With GS_4 administration, the changes in the levels of protein – bound carbohydrate components are reversed, indicating that the pathological changes secondary to diabetes is reversed by therapy.

Day *et al* (1980) found a correlation between glycosylated serum proteins and blood glucose levels in diabetic rats. In our study, a significant positive correlation is found between the blood glucose and glycosylated plasma protein levels which is reversed upon GS_4 therapy which shows that GS_4 while bringing down the glucose levels corrects the associated abnormalities. Yue *et al* (1980) found a highly significant correlation between the *in vivo* glycosylation of plasma proteins and haemoglobin and suggested that the mean glucose concentration is the most important parameter in determining the extent of protein glycosylation.

The above observations leads to the understanding that the hypoglycaemic extract of *G. sylvestre,* R. Br. Brings about blood glucose homeostasis, which in turn prevents increased glycosylation of proteins, thus reversing the onset of changes leading to micro – and macroangiopathy. Control of diabetes mellitus and the associated complications are mediated through the revival or *regeneration* of the insulin producing β-cell in the islets of Langerhans.

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LEGEND FOR FIGURES

Fig. I Blood glucose levels during oral glucose tolerance test in control (group I), uncontrolled diabetic (group II) and GS_4 treated diabetic (group III) animals.

Fig. II Blood glucose and serum insulin levels of control and experimental animals at the end of 16 weeks.

Fig. III Correlation between blood glucose and glycosylated haemoglobin of control and experimental animals. The coefficient of correlation, straight line equation and statistical significance between the two parameters in the different groups are as below:

Control (group I)	: r = 0.89, y = 1.71 x – 35.48 p 0.001
Uncontrolled diabetic (group II)	: r = 0.99 y = 2.02 x – 391. 23 p 0.001
GS_4 treated (group III)	: r = 0.95 y = 1.78 x – 67.67 p 0.001

Fig. IV Correlation between blood glucose and glycosylated plasma protein of control and experimental animals. The coefficients of correlation, straight line equation and statistical significance between the two parameters in the different groups are as below :

Control (group I)	: r = 0.99, y = 0.029 x – 1.769 p 0.001
Uncontrolled diabetic (group II)	: r = 0.97 y = 0.055 x – 16. 24 p 0.001
GS_4 treated (group III)	: r = 0.27 y = 0.011 x – 0.155 NS

NS – Not significant