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SCREENING OF ANTIDIABETIC (HYPOGLYCEMIC/ANTIHYPERGLYCEMIC) ACTIVITY OF EXTRACTS OF SPIRULINA AGAINST ALLOXAN INDUCED DIABETES IN RATS.

Panigrahi B.B.[1]*, Quamuddin M.[1], Panda P.K.[2] and Patro V.J.[3]

[1]Department of Pharmacology, HIMT College of Pharmacy, Greater Noida, G.B Nagar, Uttar Pradesh. 201310.

[2]Department of Pharmacology, UDPS, UTKAL University, Vani Vihar, Bhubaneswar, Odisha.

[3]Department of Pharmaceutical Chemistry, Roland Institute of Pharmaceutical Sciences, Behrampur, Odisha.

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***Corresponding Author**
Prof. Dr. Panigrahi B.B.
Department of Pharmacology, HIMT College of Pharmacy, Greater Noida, G.B Nagar, Uttar Pradesh. 201310.

ABSTRACT

Spirulina is a cyanobacterium, blue green algae, belongs to cyanophyceal, a prokaryotic form. Spirulina was extracted through Pet-Ether, chloroform and methanol by Soxhlet's extraction process. After making phytochemical analysis, duly confirmed through TLC proceeded for hypoglycemic activity. The antidiabetic activity of different extracts of spirulina was carried out using albino rats of either sex, diabetes was induced 12 hour fasted rats by intra peritoneal injection of alloxan. Rats with 250 mg/dl plasma glucose level were selected for experiment. Petrolium ether, Chloroform extract did not lower the blood glucose level. However Methanolic extract of Spirulina significantly reduced the blood glucose level. Standard drug used in this research study is Metformin.

KEYWORDS: Spirulina, Petrolium Ether, Chloroform, Metformin, Methanolic extract.

INTRODUCTION

Spirulina is a cyanobacterium; blue green algae belong to cyanophyceal, a prokaryotic form. The photosynthetic pigment phycocyanin and phycoerythrin makes it to be induced in

cyanophycean algal group; but the nature of its nucleus (prokaryotic) engrouped it in as a prokaryotic organism a cyanobacterium. In other words spirulina is a photosynthetic prokaryotic microorganism. It is simple microscopic blue green algae. It grows naturally in fresh, brackish, sewage water and even in saline environment. It grows through photosynthesis, hence can be termed as a vegetative food. It has already affectively promoted as a natural food.

Diabetic mellitus arises due to deficiency of insulin secretion from the β cells of the pancreas or a deficiency of the insulin action.[1] Alloxan, a cyclic urea derivative acts as a diabetogenic agent by its ability to cause selective cytotoxicity and necrosis of the β cells of the endocrine pancreas.[2] During course of diabetes the enzyme activities of gluconeogenesis with a simultaneous increase in the glycogenolytic and lypolytic pathways accompanied by a decrease in enzyme activities of glycolytic and pentose phosphate pathways.[3] Many studies have been undertaken to investigate the potential of natural medicines for the effective treatment of diabetes.[4] Although many antidiabetic agent are available, much attention has been paid recently to discover the natural product mainly due to their less toxicity and side effect; compared to non-herbal synthetic counter parts.[5,6] Ayurvedic texts also describing various antidiabetic medicine of plant product as single or in combinations and many indigenous Indian medicinal plants have been reported to be useful in managing diabetes.[6]

The molecular mechanism by which the extract improves glucose lowering and the mechanism of action are not known. Further pharmacological studies using herbal extracts invariably used either alloxan or streptozocin to induce diabetic condition.[7,8] The chemically induced diabetic animal model suffer from toxicity in different organ beside their cytotoxicity on beta cells, development of hypoglycemia due to insulin insufficiency rather than insulin resistance and difficulties in performing long term experiments due to instability of chemicals may leads to reversibility of diabetic conditions.[9] In fact sreptozotocin and alloxan have been widely used as diabetogenic agents for their ability to produce free radicals in the body, cut DNA chain in beta cells of pancreas causing necrosis, causing decreased production of insulin, leading to severe hypoinsullinemia & hyperglycemia similar to type-I diabetes in human.[10] As per the WHO(World Health Organisation) around 31.7 million individuals in India were affected diabetes during the year 2000 which may further rise to 79.4 million by the year 2030.[11] Diabetes mellitus is the chronic disorder emerging as the major health problem which increases the rate of morbidity and mortility.[12] Hypoglycemia is

the common adverse drug reaction(ADR) of the antidiabetic drugs and is associated with substantial morbidity & mortility.[12] Poor management of these two disorders leads to several complications.[13]

MATERIALS AND METHODOLOGY

Studies were carried out with reference to IAEC (Institutional Animal Ethical Committee) Regd No. 1170/ac/08/CPCSEA. Healthy Albino Rats (150-180 gm) of either sex were used. They were subjected to water *ad libitum* and diet, kept in a house with control temperature with a ratio of 12:12 hour light: dark cycle. The yield of the petroleum-ether, chloroform and methanol were subjected to chemical tests and pharmacological Screening[14-17] for antihyperglycemic activity.

Acute toxicity study/Max Tolerated Dose

Acute toxicity study was carried out as per the stair case Method[18] and also followed the OECD guide line 425.

Diabetes was induced in 12 hour fasted rats by intra-peritoneal injection of 100 mg/kg body weight of alloxan, freshly dissolved in sterile normal saline immediately before use. Measuring fasting plasma glucose after alloxan treatment assessed the diabetic state. Rats with plasma glucose level 250 mg/dl were selected for experiment. The Rats were divided into five different group and each group consist five 5 rats.

1. Control group
2. Standard (Metformin) group
3. Petroleum ether extract group
4. Chloroform extract group
5. Methanol extract group

Suspensions of each extracts were prepared by triturating each extracts with Carboxy Methyl Cellulose(CMC) sodium (0.5% w/v) gradually water was added and volume was made up to 10 ml. Then each constituent was given to respective groups through oral route. The glucose oxidase/peroxidase method was used for determination of plasma glucose level in the rats.

Screening of Hypoglycemic Activity

All extracts obtained were subjected for evaluation of hypoglycemic activity in alloxan induced diabetic rats. The data generated were subjected to one way ANOVA followed by

multiple comparision analysis using Holm-Sidak test (graph pad prism® software was used for the purpose) Metformin was taken as standard and administration of Metformin 500 mg/kg body weight showed hypoglycemic activity in alloxan induced diabetic rats as compared with control (P<0.05)

Table 10.3.2 Study of Antihyperglycemic effect of PEFS (Petrolium Ether fraction of Spirulina) on Alloxan induced Diabetes in Albino Rats.

Treatment	Blood Sugar Levels mg/dl			
	0 hr.	**1hr.**	**3hr.**	**5 hr.**
Control (Saline)	259.14±2.82	254.85±3.25	250.12±3.07	252.96±4.25
Standard (Metformin) 500 mg/kg	254.42±5.84	231.38±4.76	194.64±5.06	150.42±3.25
Pet-Ether (PEFS) Extract 200 mg/kg b.w	124.52±1.64 **50.32±2.21**	128.19±3.44 **49.80±2.11**	126.28±2.28 **48.92±2.07**	126.22±4.44 **49.77±1.87**
Pet-Ether (PEFS) Extract 300 mg/kg b.w	250.64±8.32	255.36±8.25	251.16±3.42	251.26±8.62
Pet-Ether (PEFS) Extract 400 mg/kg b.w	250.72±8.31	256.42±8.04	251.76±4.41	252.28±8.52

One way ANOVA followed by Holm Sidak test for multiple comparision analysis revealed no significant difference (p<0.05) between 400 mg/kg b.w dose and 300 mg/kg b.w. The response seen in higher dose 400 mg/kg of PEFS elicited almost equal response as shown in 300 mg/kg b.w and the lower dose 200 mg/kg b.w. shown 50.32±2.21, 49.80±2.11, 48.92±2.07 and 49.77±1.87% in 0 hr., 1 hr., 3 hr. and 5 hr. respectively. So 300 mg/kg of PEFS is the minimum dose with maximum effect is selected for comparision.

Table 10.3.3 Study of Antihyperglycemic effect of CFS (Chloroform fraction of Spirulina) on Alloxan induced Diabetes in Albino Rats.

Treatment	Blood Sugar levels mg/dl			
	0 hr.	1 hr.	3 hr.	5 hr.
Control(saline)	259.14±2.82	254.85±3.25	250.12±3.07	252.96±4.25
Standard(Metformin) 500 mg/kg	254.42±5.84	231.38±4.76	194.64±5.06	150.42±3.05
Chloroform(CFS) 200 mg/kg	233.34±3.56 **13.2±1.02**	232.61±2.65 **11.91±1.11**	128.83±3.02 **49.78±2.08**	124.36±2.23 **49.39±1.87**
Chloroform(CFS) 300 mg/kg	268.81±7.42	264.05±4.67	256.52±5.72	245.72±3.76
Chloroform(CFS) 400 mg/kg	268.86±7.41	263.62±4.36	257.03±5.36	246.25±3.68

One way AVOVA followed by Holm Sidak test for multiple comparision analysis revealed no significant difference (p<0.05) between 400 mg/kg b.w dose and 300 mg/kg b.w of CFS.

The response seen in higher dose 400 mg/kg b.w. of Chloroform fraction of Spirulina (CFS) elicited almost equal response as shown in 300 mg/kg b.w. and the lower dose 200 mg/kg b.w. shown 13.2±1.02, 11.91±1.11, 49.78±2.08, 49.39±1.87% in 0 hr., 1hr., 3 hr. & 5 hr. respectively. So 300 mg/kg of CFS is the minimum dose with maximum effect is selected for comparision.

Table 10.3.4 Study of Antihyperglycemic effect of MFS (Methanolic fraction of Spirulina) on Alloxan induced Diabetes in Albino Rats

Treatment	Blood sugar levels mg/dl			
	0 hr.	**1 hr.**	**3 hr.**	**5 hr.**
Control	259.14±2.82	254.85±3.25	250.12±3.07	252.96±4.25
Standard (Metformin) 500 mg/kg b.w.	254.42±5.84	231.38±4.76	194.64±5.06	150.42±3.05
Methanol (MFS) Extract 200 mg/kg	135.47±3.26 **49.73±1.99**	128.28±4.38 **49.99±2.06**	109.38±2.16 **49.5±1.87**	96.33±1.08 **49.99±1.68**
Methanol (MFS) Extract 300 mg/kg	269.51±7.24	256.52±8.82	218.75±3.73	192.61±1.78
Methanol (MFS) Extract 400 mg/kg	270.10±7.14	256.46±8.61	218.72±3.36	192.61±1.75

One way ANOVA followed by Holm Sidak test for multiple comparision analysis revealed no significant difference ($p<0.05$) between 400 mg/kg b.w dose and 300 mg/kg b.w. The response seen in higher dose 400 mg/kg b.w. of MFS elicited almost equal response as shown in 300 mg/kg b.w of MFS and the lower dose 200 mg/kg b.w shown in 49.73±1.99, 49.99±2.06, 49.5±1.87, 49.99±1.68% in 0hr., 1 hr., 3 hr. & 5 hr. respectively. 300 mg/kg of MFS is the minimum dose with maximum effect is selected for comparision.

Among the various extracts only the methanol extract significantly decrease blood glucose level as compared to the control group. There was 28.53% decrease in blood glucose level after fifth hour treatment of methanol extract & 40.61% decrease in blood glucose level for standard.

Other two extracts did not show significant decrease in blood glucose. Plasma glucose levels are reported in table 10.3.2 and table 10.3.3 and their graphical representations are also made regarding the same.

Table 10.3.5 Screening of Hypoglycemic activity on 1st day of Treatment to Alloxan induced Diabetes in Albino Rats

Treatment	Blood Sugar levels mg/dl % of reduction			
	0 hr	**1 hr**	**3 hr**	**5 hr**
Control(Saline)	259.14±2.82	254.85±3.25	250.12±3.07	252.96±4.25
Standard (Metformin) (500 mg/kg)	254.42±5.84	231.38±4.76 **9.06**	194.64±5.06 **23.50**	150.42±3.25 **40.88**
Pet-Ether (PEFS) Extract (300 mg/kg)	250.64±8.32	255.36±8.25**(-)**	251.16±3.42**(-)**	251.26±8.62**(-)**
Chloroform(CFS) Extract 300 mg/kg	268.81±7.42	264.05±4.67 **1.77**	256.52±5.72 **4.57**	245.72±3.76 **8.59**
Methanol (MFS) Extract 300 mg/kg	269.51±7.24	256.52±8.82 **4.82**	218.75±3.37 **18.83**	192.61±1.78 **28.53**

$p<0.05$ as compared with saline treatment group. Above values are mean ± SEM (n=5).

Table 10.3.6 Screening of Hypoglycemic Activity of different extracts on 3rd & 7th Day Treatment to Alloxan induced Diabetes in Albino Rats

Treatment	Blood sugar levels mg/dl % of reduction			
	0 hr	**1 hr**	**3 hr**	**5 hr**
Control	259.14±2.82	252.92±4.28	232.41±3.12	219.95±3.61
Standard	254.42±6.41	151.10±3.22 **40.61**	122.73±2.06 **51.76**	101.51±2.30 **60.10**
Pet-Ether (PEFS) Extract (300 mg/kg)	250.64±9.41	243.23±8.64 **2.96**	221.26±2.79 **11.72**	212.76±2.71 **15.11**
Chloroform (CFS) Extract(300 mg/kg)	268.81±7.49	255.72±3.76 **4.87**	221.36±4.63 **17.65**	206.55±3.14 **23.16**
Methanol (MFS) Extract 300 mg/kg	269.53±7.24	192.62±1.97 **28.53**	139.76±4.14 **48.15**	109.27±2.29 **59.46**

$p<0.05$ as compared with saline treatment group. Above values are mean ± SEM (n=5).

Effect of different Extracts of blood glucose level on 3rd & 7th Day

Different extracts were administered for 7 days to observe any significant effect on blood glucose level. It was observed that pet. Ether, Chloroform extracts did not lower the blood glucose level significantly tested on 3rd and 7th day. Treatment with Methanolic extract showed the reduction in blood glucose level by 59.46 % on 7th day.

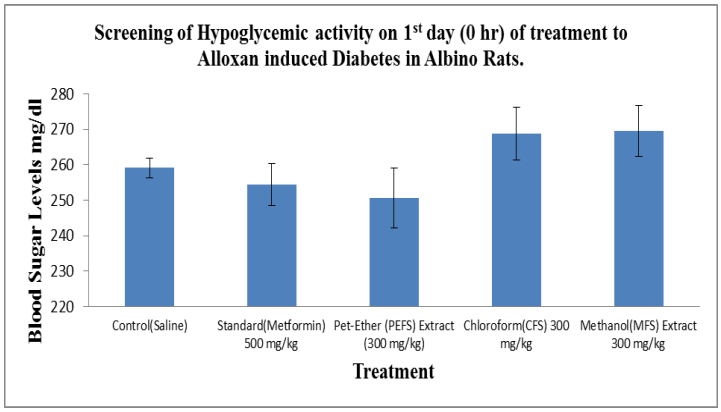

Figure 10.3.2(a) Screening of Hypoglycemic activity on 1st day (0 hr) of treatment to Alloxan induced Diabetes in Albino Rats.

Values are mean ± SEM (n=5) $p<0.05$.

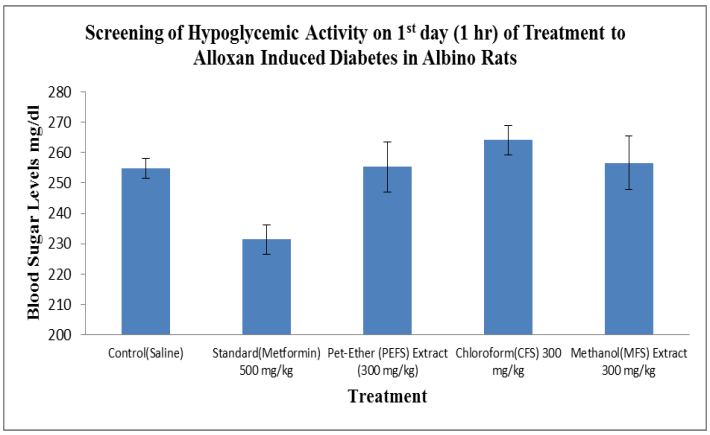

Figure 10.3.2 (b) Screening of Hypoglycemic Activity on 1st day (1 hr) of Treatment to Alloxan Induced Diabetes in Albino Rats

Values are mean ± SEM (n = 5) $p< 0.05$.

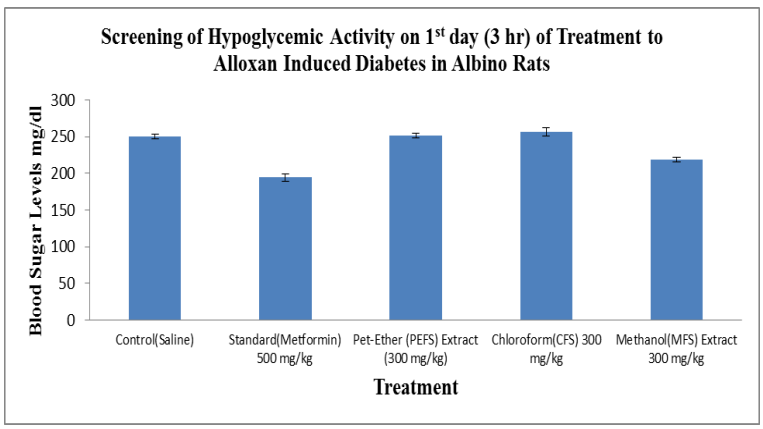

Figure 10.3.2 (c) Screening of Hypoglycemic Activity on 1st day (3 hr) of Treatment to Alloxan Induced Diabetes in Albino Rats.

Values are mean ± SEM (n = 5) $p < 0.05$.

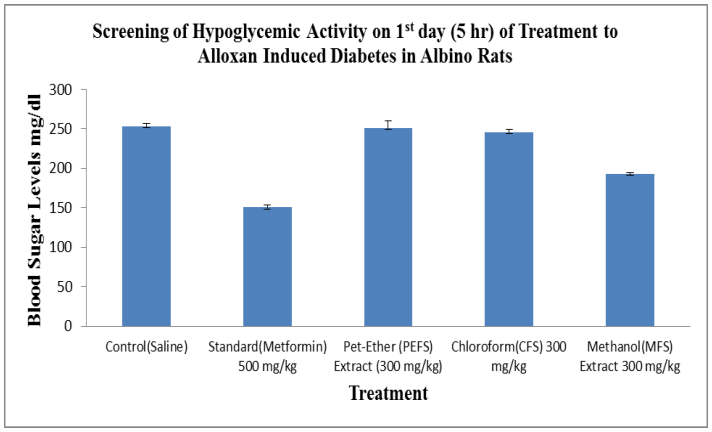

Figure 10.3.2 (d) Screening of Hypoglycemic Activity on 1st day (5 hr) of Treatment to Alloxan Induced Diabetes in Albino Rats

Values are mean ± SEM (n = 5) p< 0.05.

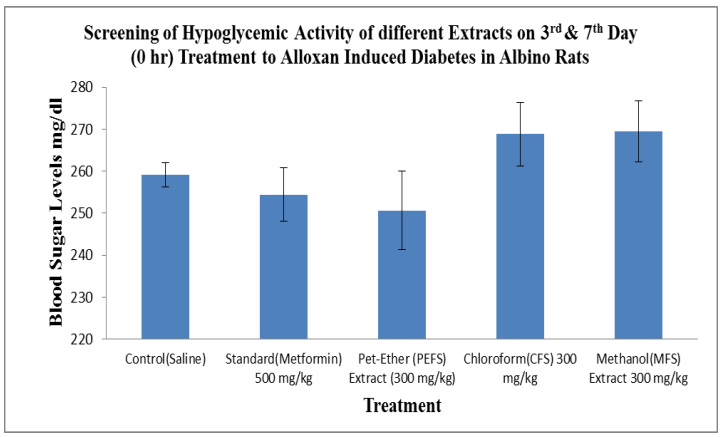

Figure 10.3.3(a) Screening of Hypoglycemic Activity of different Extracts on 3rd & 7th Day (0 hr) Treatment to Alloxan Induced Diabetes in Albino Rats

Values are mean ± SEM (n=5) p < 0.05

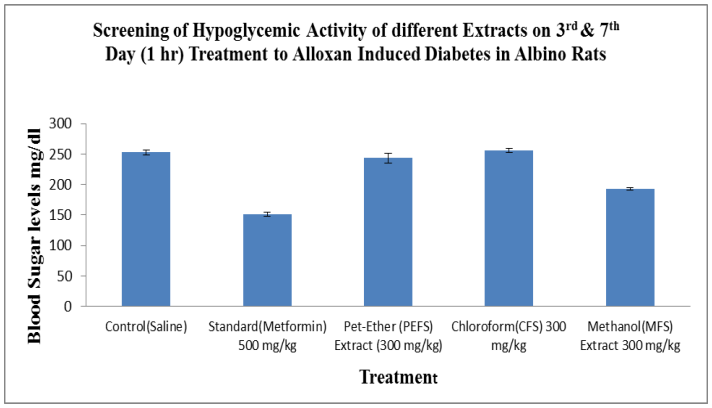

Figure 10.3.3(b) Screening of Hypoglycemic Activity of different Extracts on 3rd & 7th Day (1 hr) Treatment to Alloxan Induced Diabetes in Albino Rats

Values are mean ± SEM (n=5) p<0.05.

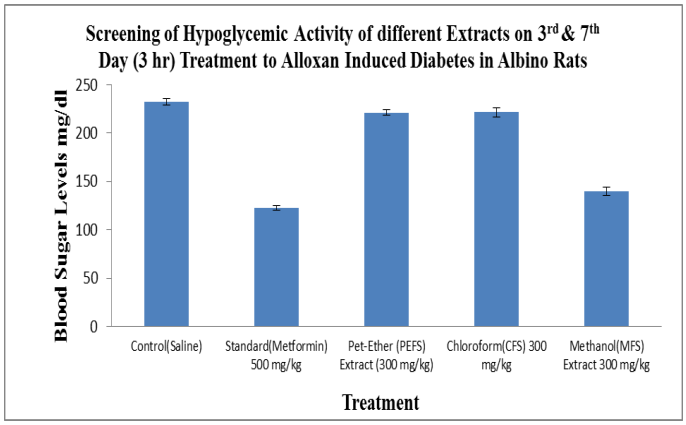

Figure 10.3.3 (c) Screening of Hypoglycemic Activity of different Extracts on 3rd & 7th Day (3 hr) Treatment to Alloxan Induced Diabetes in Albino Rats

Values are mean ± SEM (n=5) $p<0.05$.

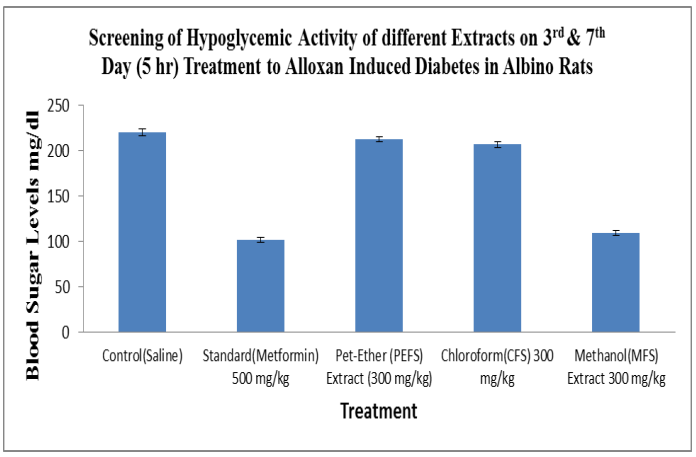

Figure 10.3.3 (d) Screening of Hypoglycemic Activity of different Extracts on 3rd & 7th Day (5 hr) Treatment to Alloxan Induced Diabetes in Albino Rats

Values are mean ± SEM (n=5) $p<0.05$.

RESULTS

In the present study Petroleum Ether extract, Chloroform extract and Methanolic extract of spirulina were subjected to evaluation of hypoglycemic activity. Among which the Methanol extract had the ability to lower the blood sugar level significantly. So further study on methanol extract of spirulina was carried on.

DISCUSSION

In this study diabetes was induced in 12 hour fasted rats by intra-peritoneal injection of 100 mg/kg body weight of Alloxan. Alloxan produced oxygen free radicals which cause pancreatic injuries and could be responsible for increased blood sugar level in the rats.

Alloxan a cytotoxic agent induces chemical diabetes (alloxan diabetes) in various animal species through destruction of islets of langerhans of the pancreas, following which there is a formation of redox cycle for generation of reactive oxygen species (ROS), superoxide radicals and hydrogen peroxide.[19] From the preliminary phytochemical analysis it is clear that tannic acid is a potent inhibitor of glucose uptake which produced a marked loss in glucose transport capacity in isolated rat intestinal brush border membrane vesicles by dissipation of the sodium ion electrochemical gradient that provides the driving force for active glucose accumulation.[20] Plants that contain the active principles such as glycoside and flavonoids have anti-oxidant activity and are claimed to possess anti-diabetic effect.[21] The anti-diabetic activity of ethanolic extract of leaves of *punica granatum* may be due to presence of phytochemicals (flavonoids, tannins, glycosides) in it, which are strong antioxidants.[22] Moreover flavonoids are known to regenerate the damaged β cells in alloxan induced diabetic rats.[23] Amongst the extracts only methanol extract significantly decreases the blood glucose level as compared to control group was 28.53% decrease in blood glucose level after fifth hour treatment of methanolic extract and 40.61% decrease in blood glucose level for standard (metformin) other two extracts i.e Petrolium ether extract and Chloroform extract did not show significant decrease in blood glucose level. It is because MFS contains Tannins, Flavones and Flavonoids Glycosides which have been confired through phyto-chemical analysis duly confirmed through TLC.

CONCLUSION

Hence it is concluded that methanolic fraction of spirulina has got significant antidiabetic effect in comparision to Petroleum Ether fraction and Chloroform fraction of spirulina in albino rats. Further research on isolation and characterization of active phytoconstituents present in the methanolic fraction in the future interest.

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