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Anti-Diabetic and Anti-Obesity Effect of a Crude Polysaccharide Isolated from *Vitex negundo* Linn. Leaves in db/db Mice

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Abstract: Various plant actives have been reported for their therapeutic potential in obesity so far and plant polysaccharides are one of them. Naturally occurring non-starch polysaccharides are widely known for their anti-oxidant, immunomodulatory, anti-diabetic and anticancer activities as well in various *in vitro* and *in vivo* test systems. This paper focuses on one such crude polysaccharide fraction isolated from the leaves of the plant *Vitex negundo* which was tested for its anti-obesity activity in male db/db mice at 50 mg/Kg dose via both subcutaneous and per oral route of administration for 7 days. The activity via both the routes was compared with pair-fed and normal control animals for various parameters such as daily food intake, body weight, fasting serum glucose, insulin and triglyceride levels as well as changes in glucose levels during oral glucose tolerance test (OGTT). Obtained results demonstrate a significant reduction in food intake, body weight and fasting glucose levels in crude polysaccharide treated animals which are suggestive of its anti-obese activity. Further, a remarkable increase in fasting insulin and a significant decrease in glucose intolerance in crude polysaccharide treated animals was also observed, which indicate its anti-diabetic activity. Thus, this study primarily establishes a positive role of the crude polysaccharide from *V. negundo* leaves in treatment of both obesity and type 2 diabetes mellitus.

Keywords: crude polysaccharide, *Vitex negundo*, obesity, type 2 diabetes, db/db mice, inflammation, homeostatic model assessment (HOMA), area under the curve (AUC).

INTRODUCTION

Type 2 diabetes mellitus, which is a metabolic disorder, is associated with weight gain and increased body mass index (BMI) in people over age of 40 years. Insulin resistance is a key phenomenon in both obesity and type 2 diabetes mellitus. It is characterized by fasting hyperinsulinemia and hyperglycemia, postprandial hyperglycemia, hyperlipidemia, impaired glucose tolerance and elevated inflammatory markers. In case of obesity induced type 2 diabetes mellitus, hyperglycemia and hyperlipidemia also results in generation of free radicals and reactive oxygen species (ROS), which in turn triggers inflammatory response, reduces insulin sensitivity and leading to degeneration of beta cell mass as well due to oxidative stress. Usually, insulin resistance occurs when pancreatic beta cells try to produce more amount of insulin to control hyperglycemia [1,2].

V. negundo has been traditionally used as anti-oxidant, analgesic, anti-inflammatory, as a vermifuge and in arthritis [3] and leaves are reported for their antibacterial, antitumor, astringent, febrifuge, sedative, tonic and vermifuge properties [4]. Various constituents of the plant include flavanoids, flavone glycosides, volatile oil, triterpenes, tannins and polysaccharides [4, 5]. Moreover, anti-inflammatory and anti-diabetic potential of the plant leaves has also been reported viz. mature fresh leaves of *V. negundo* were confirmed for anti-inflammatory, analgesic and antihistaminic activities in various inflammation models [6]; water extract of *V. negundo* showing test for polysaccharide positive, demonstrated anti-inflammatory activity in formalin induced inflammation [5]; aqueous and ethanolic leaf extracts of *V. negundo* (500 mg/Kg) demonstrated significant antidiabetic potential in alloxan induced diabetes model of rats [7] and methanolic extract of the *V. negundo* leaves exhibited anti-hyperglycemic activity post glucose challenge in fasting mice [8].

Further, naturally occurring polysaccharides are widely reported for their immunomodulatory, anti-cancer, anti-oxidant, anti-inflammatory and anti-hyperglycemic potential [9, 10]. Many isolated crude polysaccharides from different parts of various plants have been reported for their antihyperglycemic activities and ability to reduce insulin resistance; to

name a few - crude polysaccharides isolated from the root of *Liriope spicata* var. *prolifera* demonstrated hypoglycemic and hypolipidemic activities in normal and STZ induced type 2 diabetes mellitus [11]; crude polysaccharides from endodermis of *Citrus paradisi* demonstrated inhibitory effects on α-glucosidase and α-amylase enzymes [12]; galactomannans isolated from *Trigonella foenumgraecum* L. seeds showed anti-hyperglycemic activity in alloxan induced diabetes in mice in both acute and chronic studies [13]; a sulphated polysaccharide isolated from *Saccharina japonica* has been reported for hypoglycemic activity in alloxan induced diabetic rats and mice [14] and polysaccharide from lotus plumule (*Nelumbo nucifera Gaertn*) has been reported for promoting insulin release and ameliorating lipid profile and glucose intolerance in non-obese diabetic mice [15]. Some naturally occurring polysaccharide are also reported for their lipid lowering potential and usefulness in obesity to name a few - *Ulva lactuca* polysaccharides (ULPS) are reported to inhibit *α*-amylase by 53% and 34% and maltase by 97 and 164% respectively in both plasma and small intestine, thereby reducing blood glucose levels and inhibit lipase activity in plasma and small intestine by 235 and 287% respectively, thereby lowering plasma LDL and triglyceride levels in diabetic rats [16]; polysaccharides isolated from the mushroom *Pleurotus tuber-regium* demonstrated hypoglycemic and hypolipidemic activities via upregulation of liver PPAR-α levels and additionally it also restored HDL levels in diabetic-obese rats [17] and polysaccharides isolated from the water extract of mushroom *Ganoderma lucidum* reduced body weight, inflammatory cytokines, insulin resistance in high fed diet induced obese mice [18].

Considering the anti-diabetic potential of *V. negundo* leaves and therapeutic role of crude plant polysaccharides in obesity as well as type 2 diabetes mellitus from above reports, this paper aims to study the effect of crude polysaccharide isolated from the leaves of *V. negundo* in male db/db mice which serve as a model for both obesity and type 2 diabetes mellitus. In this experimentation, SC route study was performed first, since we were exploring the therapeutic property of the crude polysaccharide of *V. negundo* for the first time and due to probability of the polysaccharides to degrade in the acidic environment when administered enterally, PO route was avoided in first instance. Moreover, since any new compound might trigger an inflammatory response when given SC, we didn't evaluate inflammatory biomarkers for SC study which may lead to difficulty in concluding whether the effect (if at all found) on inflammation is due to test compound or due to allergy. However, since the research findings as mentioned previously in this section mentions use of PO route of administering crude polysaccharides in their studies and suggesting the possibility of them getting absorbed (due to their low molecular weights) orally and eliciting therapeutic effect, we also explored PO route in our second study with the same evaluation parameters as of SC route study in addition to the inflammatory cytokines viz. TNF-α and IL-6.

MATERIALS AND METHODS

Animals

10-11 weeks old male db/db mice were used for the study which were approved IAEC. db/db is a very well reported mouse model for obesity, diabetes and dyslipidemia. These animals are leptin resistant due to point mutation in leptin gene. They are also useful in immunology, inflammation and metabolism research. They become obese at around 3-4 weeks of age and elevation of plasma insulin begins at 10-14 days of age while that of plasma glucose occurs at 4-8 weeks. Moreover, they are polyphagic, polydipsic and polyuric in nature. All of these characteristics make them useful animal model in type 2 diabetes induced obesity type of condition [19].

Study groups

Mice were randomized in three study groups for each of the route used in the study ie per oral (PO) and subcutaneous (SC) - vehicle treated, polysaccharide treated and pair fed of polysaccharide, wherein, pair fed received only vehicle and no polysaccharide treatment. The purpose of pair-fed group here was to differentiate the effect of crude polysaccharide on various evaluation parameters to that of polysaccharide treated group. Each study group for both the studies consisted of 6 animals which were housed in polypropylene cages at standard conditions *ad libitum*.

Test compound – crude polysaccharide fraction from *V. negundo* leaves

Crude polysaccharide fraction was isolated from the leaves of *V. negundo* Linn. by hot water extraction and methanol precipitation was as per reported methods [20]. It was administered subcutaneously (S.C.) and per orally (PO) to polysaccharide treated group at dose of 50 mg/Kg twice a day for 7 consecutive days. Vehicle system used for suspending the drug was PEG-400:Tween80:NaCMC (0.1:0.1:0.5) in water.

Evaluation parameters

For both the study routes, evaluation parameters such as body weight, feed intake, fasting serum glucose, insulin and triglycerides were measured after 7 days of treatment with crude polysaccharide fraction ie ~16 hours after the last dose. In PO crude polysaccharide treated animals, additionally, serum TNF-α and IL-6 were measured after 7 days of crude polysaccharide treatment.

Cumulative feed intake

(g)/animal was recorded for all the study groups every day post first day of polysaccharide treatment.

% Change in body weight

Daily body weight of animals in each group was recorded right from day 0 and % change in body weight was calculated for all the days of treatment. Treatment was started immediately after 0 day recording.

Fasting serum insulin and glucose

Both glucose and insulin in serum samples were measured post 16 hours of fasting after the last dose at day 7 using commercially available kits. Fasting insulin was measured from the pool of six serum samples from each group.

HOMA-IR and HOMA-β

Homeostatic model of assessment (HOMA) is a method to evaluate beta cell function (HOMA-β) and insulin resistance (HOMA-IR) using fasting glucose and insulin concentrations. Both types were calculated on day 7 as follows [21]:

HOMA-IR= {Fasting insulin (mIU/L)×Fasting glucose (mmol/L)} / 22.5

HOMA-% beta={(20x Fasting insulin (mIU/L)/ (Fasting glucose (mmol/L-3.5)}%

Fasting serum triglycerides

Fasting serum serum triglycerides were measured at day 7 from all study groups using commercially available kit.

Oral glucose tolerance test (OGTT) and Area under the curve (AUC)

Oral glucose tolerance test (2g/Kg) was also carried out after 7 days of treatment and area under the curve (AUC) was calculated for the same. The oral glucose tolerance test (OGTT) measures the body's ability to utilize glucose, which is the body's main source of energy. A glucose solution (2g/kg) was orally administered to overnight fasted mice. Blood samples were collected via retro-orbital puncture at 0 (before glucose loading), and after 15, 30, 60, 120 and 240min of oral glucose administration, serum samples were separated and analyzed for glucose concentrations using commercially available kits.

Fasting serum TNF-α and IL-6

Inflammatory cytokines specific for type 2 diabetes and obesity viz. TNF-α and IL-6 [22] were measured from fasting serum samples on day 7 after the last dose in PO polysaccharide group.

Statistical analysis

All the values were expressed as mean ± SEM for calculation (except where not mentioned) and the significant difference was determined using one way or two way ANOVA test (wherever applicable) with post tests and the value of p less than 5 % ($p < 0.05$) was considered as statistically significant.

RESULTS

Effect of crude polysaccharide treatment on cumulative food intake in male db/db mice

In comparison to vehicle treated group, a significant drop in feed intake was observed from day 2 onwards in SC polysaccharide treated group (Figure 1(a)) and from day 4 onwards in PO polysaccharide treated group (Figure 1(b)). Since pair-fed group of polysaccharide received the same amount of feed as that of polysaccharide treated group, it is not included in the graph.

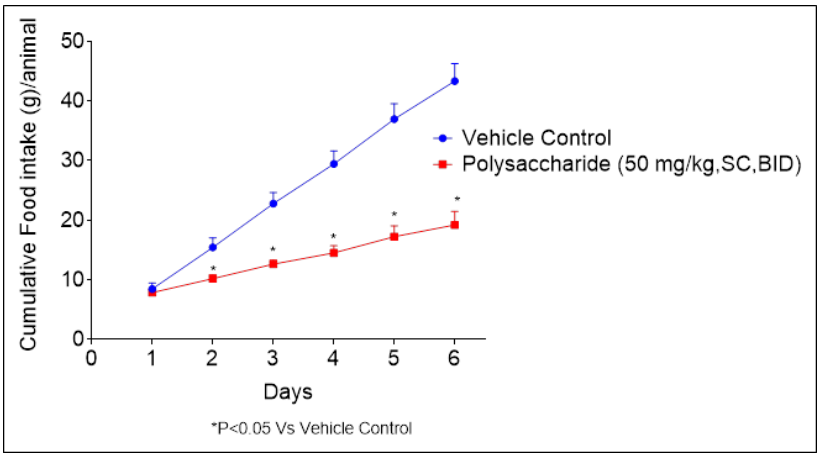

Fig-1(a): A plot of cumulative food intake (g)/animal vs days for SC route

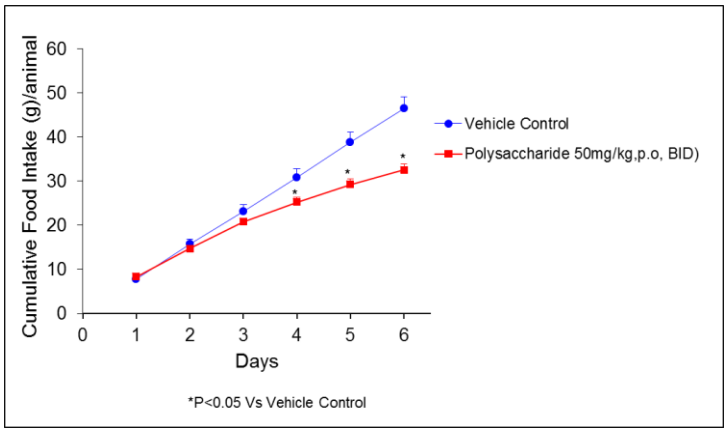

Figure 1(b): A plot of cumulative food intake (g)/animal vs days for PO route

Effect of crude polysaccharide treatment on %change in body weight in male db/db mice

In comparison to vehicle control group, a significant decrease in %change in body weight in animals treated with SC polysaccharide was evident day 3 onwards (Figure 2(a)) and in PO polysaccharide treated animals, it was day 2 onwards (Figure 2(b)).

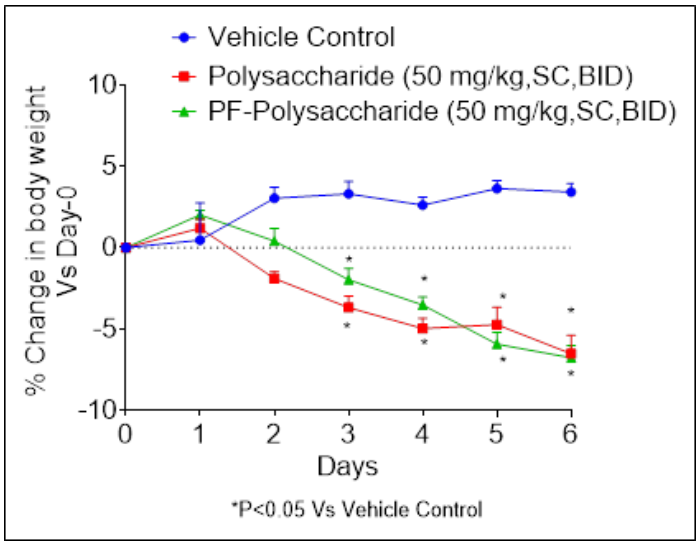

Fig-2(a): A plot of %change in body weight vs days for SC route

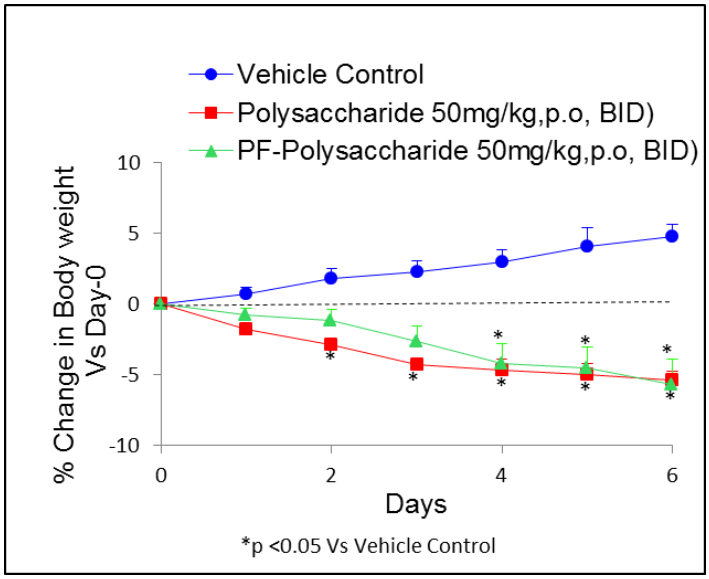

Fig-2(b): A plot of %change in body weight vs days for PO route

Effect of crude polysaccharide treatment on fasting glucose and insulin in male db/db mice

Fasting glucose concentrations reduced significantly in SC polysaccharide as well as PO polysaccharide treated animals in comparison to the vehicle control post 7 days of treatment which approximated to almost 50% (Figure 3(a)). Fasting insulin, on the other hand, increased by almost 5 folds in both SC and PO polysaccharide treated group in comparison to the vehicle control (Figure 3(b)).

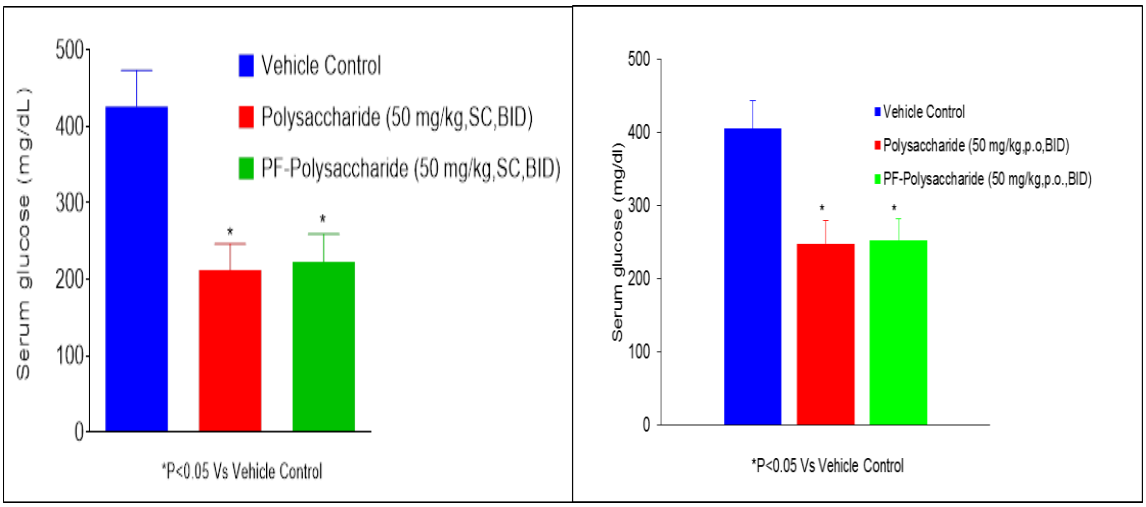

Fig-3(a): A plot of fasting serum glucose vs study groups at day 7 for SC and PO routes

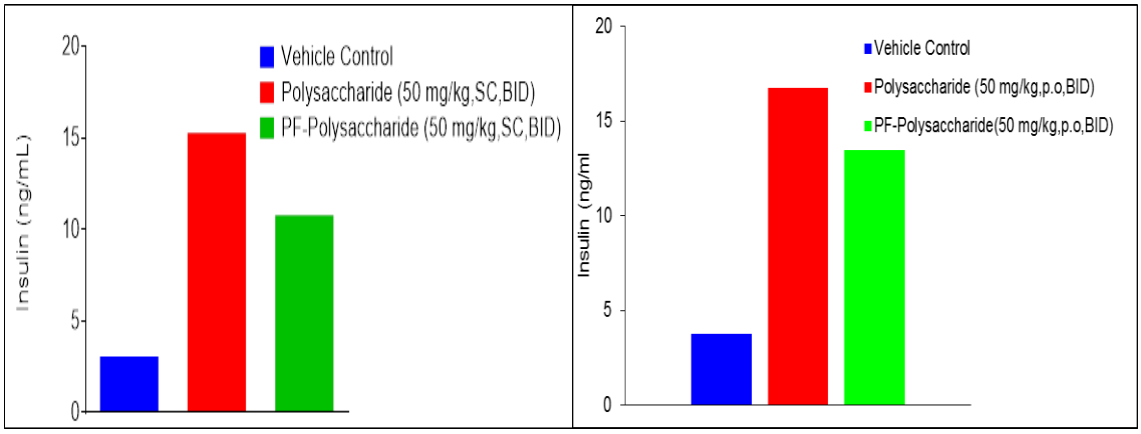

Fig-3(b): A plot of fasting serum insulin vs study groups at day 7 for SC and PO routes

Effect of crude polysaccharide treatment on HOMA-IR and HOMA β in male db/db mice

HOMA-IR and HOMA-β were increased in SC polysaccharide treated group by 2.5 and 12.1 folds respectively in comparison to vehicle control (Table 1 (a)). In case of PO polysaccharide treated group, HOMA-IR and HOMA-β increased by 2.73 and 8.23 folds respectively in comparison to vehicle control (Table 1 (b)).

Table-1(a): HOMA-IR and HOMA-β at day 7 for SC route

Treatment group	HOMA-IR	Fold Change Vs Vehicle Control	HOMA-beta	Fold Change Vs Vehicle Control
Vehicle Control	91.1	-	87.8	-
Polysaccharide (50 mg/kg,SC,BID)	225.9	2.5	1065.8	12.1
PF-Polysaccharide (50 mg/kg,SC,BID)	167.3	1.8	700.2	8.0
Data represents Mean±SEM(n=6) Except n=5 in Polysaccharide (50 mg/kg,SC,BID)				

Table-1(b): HOMA-IR and HOMA-β at day 7 for PO route

Treatment group	HOMA-IR	Fold Change Vs Vehicle Control	HOMA-beta	Fold Change Vs Vehicle Control
Vehicle Control	105.4		114.0	
Polysaccharide (50 mg/kg,p.o.,BID)	287.7	2.73	938.7	8.23
PF-Polysaccharide (50 mg/kg,p.o.,BID)	235.8	2.24	735.8	6.45
Data represents Mean±SEM(n=6)				

Effect of crude polysaccharide treatment on fasting triglycerides in male db/db mice

Unexpectedly, fasting triglycerides levels were significantly increased in SC polysaccharide treated group in comparison to vehicle control (Figure 4 (a)). In case of PO treated group, no such significant change was observed (Figure 4(b)).

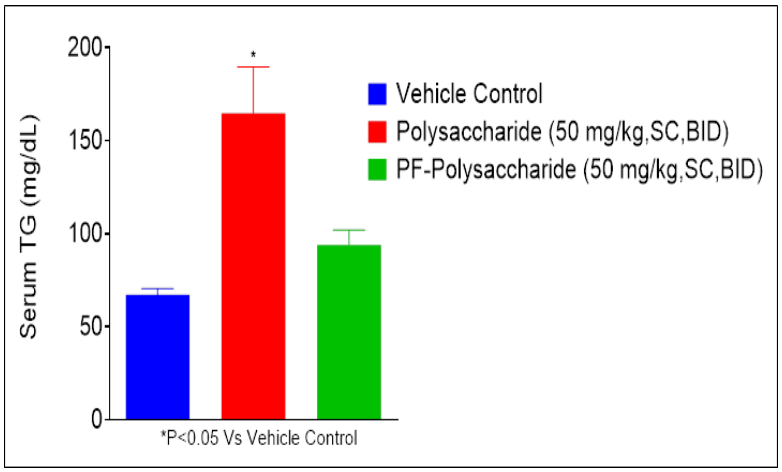

Fig-4(a): A plot of fasting serum triglycerides (TG) vs study groups at day 7 for SC route

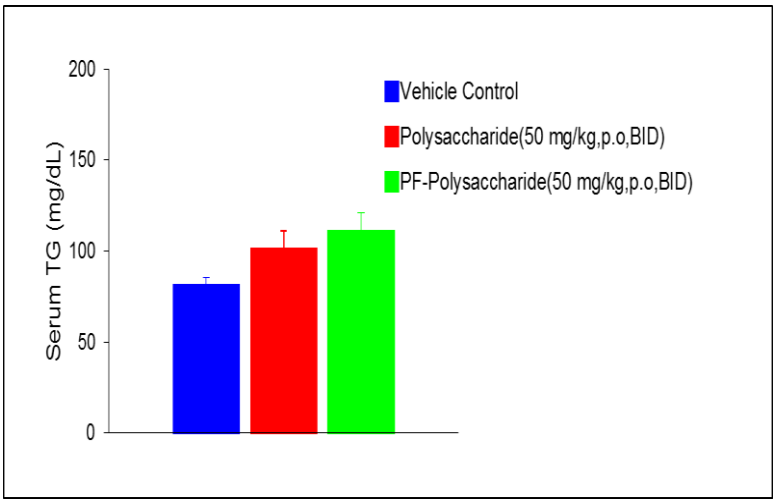

Fig-4(b): A plot of fasting serum triglycerides (TG) vs study groups at day 7 for PO route

Effect of 7 days crude polysaccharide treatment on OGTT and AUC in male db/db mice

A significant reduction in AUC was observed in both SC and PO polysaccharide treated group in comparison to the vehicle control. Moreover, a significant reduction in serum glucose both before and after glucose load (2g/Kg, PO) ie. at 0, 15, 30, 60, 120 and 240 min was also evident in SC and PO polysaccharide treated group in comparison to vehicle control (Figure 5(a) and (b)).

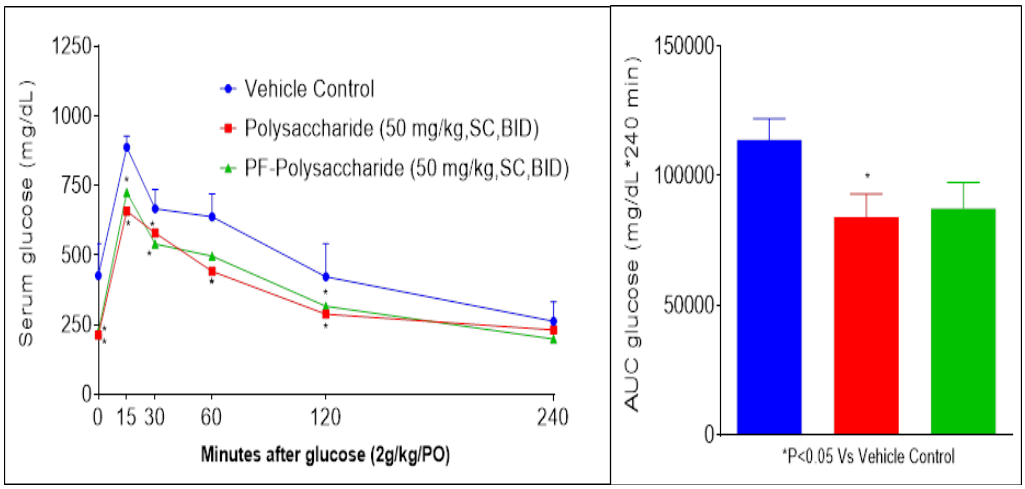

Fig-5(a): A plot of serum glucose levels vs time points post OGTT at day 7 and AUC representation of the same for SC route

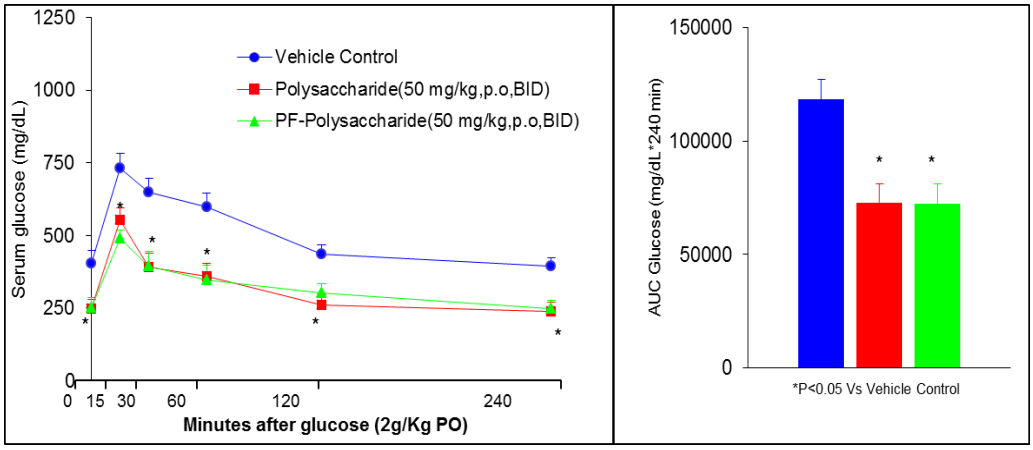

Fig-5(b): A plot of serum glucose levels (mg/dl) vs time points OGTT at day 7 and AUC representation of the same for PO route

Effect of 7 days crude polysaccharide treatment on fasting serum TNF-α and IL-6 in male db/db mice for PO route

A remarkable though non-significant decrease in both TNF-α and IL-6 was observed in PO polysaccharide treated group in comparison to vehicle control (Figure 6).

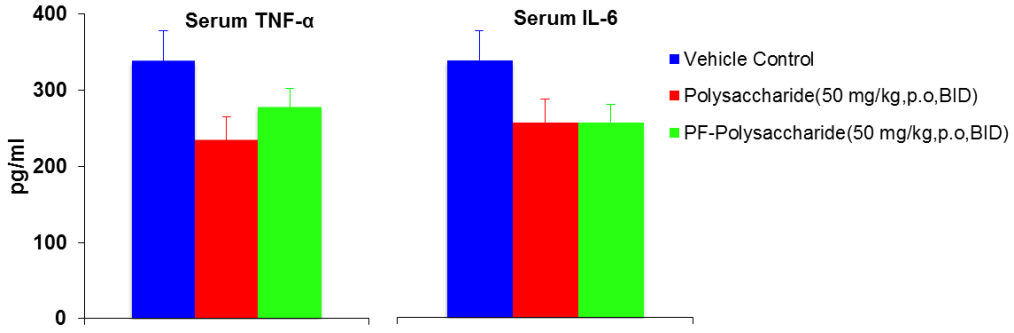

Fig-6: A plot of serum TNF-α and IL-6 (pg/ml) at day 7 for PO route

DISCUSSION

Crude polysaccharide which was isolated from aqueous extract of *V. negundo* leaves was evaluated for its effect on various parameters such as daily change in feed intake and body weight, fasting serum glucose, insulin and triglycerides levels as well as oral glucose tolerance test (OGTT) and area under curve (AUC) at the end of 7 days of treatment via SC and PO routes. Pair-fed group was used in this experiment for routes – pair-fed means same quantity of feed given to two study

groups to check and confirm if the effect (if observed any), is due to the test component administered (here, crude polysaccharide of *V. negundo* leaves). Since hyperphagia results in obesity and db/db mouse is a model for obesity too, in this study, daily feed intake in g/animal for each group was measured. Both SC and PO polysaccharide treated animals exhibited a significant reduction in cumulative feed intake in comparison to the vehicle control which group which did not receive polysaccharide treatment, SC being the earlier in showing the effect than PO route, suggesting appetite suppressant properties of the polysaccharide. Moreover, a significant reduction in %change in body weight was also observed day 3 onwards in SC polysaccharide treated animals as compared to the vehicle group which correlates with the reduction in cumulative feed intake observed on day 2 for the same route. Such correlation was not observed in PO route, however, both cumulative feed intake and %change in body weight were reduced significantly. Elevated fasting and post-prandial glucose levels are characteristic features of type 2 diabetes mellitus. In this study, a significant decrease in fasting serum glucose and area under curve (AUC) post oral glucose tolerance test (OGTT) were seen in 7 days SC and PO polysaccharide treated animals as compared to vehicle control, which are suggestive of anti-diabetic effect of the *V. negundo* leaf polysaccharide. Fasting serum glucose levels in pair-fed polysaccharide group were also significantly reduced even though it received only vehicle which might be due restricted feed given since it was kept in pair-fed situation with polysaccharide treated group. Moreover, almost 5 fold increase in fasting serum insulin levels after 7 days in SC and PO polysaccharide treated group in comparison to vehicle control group, is suggestive of the insulin releasing effect of the *V. negundo* leaf polysaccharide. Further, increase in HOMA-IR and HOMA-%β for SC and PO polysaccharide group is due to insulin releasing and beta cell sensitizing properties of the polysaccharide respectively for both HOMA types. This suggests, due to elevated insulin levels, there is glucose utilization and increased beta cell sensitivity, hence improved glucose intolerance in SC as well as PO polysaccharide groups. Triglycerides are an important link in development of insulin resistance. Fasting serum triglycerides were unexpectedly increased post 7 days of SC polysaccharide treated mice in comparison to vehicle control which might be possible due to higher circulating insulin levels and lipid mobilizing effect of the polysaccharide which might get stabilized upon longer duration of treatment. No such significant change was observed in PO treatment. Serum TNF-α and IL-6, which are characteristic inflammatory cytokines involved in both obesity and diabetes, were remarkably and non-significantly decreased in PO polysaccharide group, suggesting a moderate anti-inflammatory potential of the *V. negundo* leaf polysaccharide.

CONCLUSIONS

V. negundo leaf polysaccharide demonstrated a significant reduction in body weight, feed intake, fasting glucose and AUC patterns via both SC and PO routes in db/db mice. It also improved impaired glucose tolerance and beta cell sensitivity via insulin release property as evident from the obtained results. In addition to these metabolic effects, it also possesses a considerable anti-inflammatory potential. However, its safety and efficacy for chronic treatment need to be studied. Overall, in acute study, *V. negundo* leaf polysaccharide was found safe, tolerable and efficacious for *in vivo* conditions of type 2 diabetes and obesity along with mild anti-inflammatory property.

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Conflict of interest

There is no conflict of interest.

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