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Bacoside-A diminishes liver functional enzymes and improves carbohydrate metabolic key enzymes in streptozotocin a rat model of T2DM

Shanmugam Devi bala [a], Ramalingam Saravanan [a,b,*]

[a] Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, 608002, Tamil Nadu, India
[b] Department of Biochemistry, Government Arts and Science College, Kumbakonam, Tamilnadu, India

A B S T R A C T

Type 2 diabetes mellitus (T2DM) is a global alarming predominant metabolic disease than other diabetes and incurs substantial burden to public and healthcare systems. Our study is designed to endeavor the valuable effect of bacoside-A on hepatic key enzymes of carbohydrate metabolism in streptozotocin (STZ) induced diabetic rats. A single intraperitoneal (i.p) injection of STZ (40 mg/kg body weight [BW]) is administered to adult male Wistar rats for the attainment of diabetes. Diabetic rats were orally treated with bacoside-A of various doses (5, 10 and 20 mg/kg BW) for 45 days. Bacoside-A (10 mg/kg BW) showed more pronounced effect than the other doses and thus resulted in a significant reduction in the level of plasma glucose, glycosylated hemoglobin (HbA1c) and an rise in insulin and hemoglobin levels. Administration of bacoside-A showed significant elevated in the activity of glycolytic enzyme (hexokinase) and hepatic shunt enzyme (glucose-6-phophate dehydrogenase) whereas significant decline in the activity of gluconeogenic enzymes (glucose-6-phosphatase and fructose-1,6-bisphosphatase) in diabetic rats. Further, bacoside-A administration to diabetic rats enhanced body weight, liver glycogen content and modulating its metabolizing enzymes (glycogen synthase and glycogen phosphorylase) demonstrated the antihyperglycemic potential of bacoside-A in diabetic rats. Furthermore, hepatic marker enzymes such as aspartate transaminases (AST) and alanine transaminases (ALT) activities significantly decreased in diabetic rats and tend to normalcy in bacoside-A treated diabetic rats. Glibenclamide, a reference drug. In conclusion, our data explicated the bacoside-A, a promising antidiabetic agent which infers in modulating hepatic key enzymes by preventing defect in carbohydrate metabolism against T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is considered as an outbreak of epidemic disease and apprehending an alarming global health issue. T2DM is initiated by multifarious biological factors such as impairment in pancreatic insulin secretion, malfunctioned carbohydrate metabolism, resistance of peripheral tissues to the glucose-utilizing effect of insulin, and amplified glucose production in liver (Toma et al., 2019; Li et al., 2015). The International Diabetes Foundation (IDF) estimates that 425 million populations were affected by diabetes worldwide in 2017 and this figure may probable to rise 629 million by the year 2045 with most of the upturn from developing countries (IDF,2017). India is home to the prevalent number of diabetics in the world, 40.9 million diabetic cases in 2007 and these numbers are predicted to 69.9 million within the year 2025 (Tharkar et al., 2010).

Liver is principal organ for glucose reserve in the body additionally it serve as an insulin-dependent tissue that plays a central role in blood glucose homeostasis. Hence, liver assents to garner the superfluous blood glucose and to demobilize glucose in hypoglycemic states. Defective postprandial insulin secretion leads to hyperglycemia and subsequent downgrade in insulin sensitivity (Saini, 2010). Deterioration of insulin control aggravates metabolic disturbances by modifying hepatic key enzyme activities in the diabetic condition. Inclusively these progressions switch to endamage peripheral glucose utilization and expand hepatic glucose production (Li et al., 2011). Consequently, hepatic glucose metabolic regulators are taken into consideration of tremendous targets in the management of T2DM. Hyperglycemia occurring in diabetes does not only damage cellular proteins, membrane lipids and nucleic acids, however boost up the rate of onset of disease complications. Sustentation of normoglycemia includes the integration and synchronized regulation of numerous metabolic pathways including gluconeogensis and glycolysis.

Owing to the adverse features of the predictable remedies for diabetes, attention on exploring natural remedy is a definitive target. The priority of our study is to seek therapeutic and preventive strategies which might decelerate the causative processes of T2DM and improve the management of diabetes associated complications. Considering the heterogeneity of T2DM, current therapies are often limited. Therefore, the survey of new phytocompounds with anti-diabetic action is of principally important.

* Corresponding author. Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar, 608002, Tamil Nadu, India.
E-mail addresses: saranbio@rediffmail.com, jayamsaranbio@gmail.com (R. Saravanan).

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Bacoside-A is triterpenoid saponin, mainly isolated from *Bacopa monnieri* (Garai et al., 1996) and used as conventional ayurvedic medicine for treating various diseases. Numerous studies revealed that bacoside-A possesses potent antioxidant, anticarcinogenic (Janani et al., 2010), hepatoprotective and wound healing activities. Ghosh et al. (2008) reported that ethanolic extract of *Bacopa monnieri* shown to have antidiabetic properties. General literature survey represents that there is no available scientific reports on the beneficial properties of bacoside-A on animal model of diabetes. Therefore, the current study is designed to evaluate the antidiabetic effect of bacoside-A in the STZ induced experimental diabetic rats.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Bacoside-A was purchased from Natural Remedies Private Limited, Bangalore, India. Standard pellet diet was purchased from Hindustan Lever Ltd, Bangalore-560066, India. All other chemicals and solvents used in this study were of analytical grade and were purchased from Hi Media (Mumbai, India) and SD-Fine Chemicals (Mumbai, India).

2.2. Experimental animals

About 180–220 g of adult male albino Wistar rats were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. All rodents were reared properly in clean, sterile, polypropylene cages under standard vivarium conditions (12 h light/dark cycles). Standard laboratory diet ad libitum along with water were provided. The experiments were carried out by following strictly the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Reg No.160/1999/CPCSEA, Proposal. No. 1124).

2.3. Induction of experimental diabetes mellitus

T2DM was induced overnight fasted rats by a single i.p. infusion of STZ (40 mg/kg body weight) dispersed in freshly prepared citrate buffer (0.1 M, pH 4.5). STZ induced rats were permitted to drink 20% glucose solution throughout the night to overcome the initially drug-induced hypoglycemic mortality (Ramachandran and Saravanan, 2013). The induction of T2DM in rats was affirmed by evaluating the elevated plasma glucose levels, 72 h post STZ injection. Rodents with fasting plasma glucose levels in excess of 250 mg/dl were act as diabetic and chosen for the study.

2.4. Experimental design

A total number of 42 rats were randomly grouped into seven, each consisting of six animals (n = 6), Group 1: Normal Control, Group 2: Normal + Bacoside-A (20 mg/kg BW), Group3: Diabetic control, Group 4: Diabetic + Bacoside-A (5 mg/kg BW), Group5: Diabetic + Bacoside- A (10 mg/kg BW), Group6: Diabetic + Bacoside-A (20 mg/kg BW), Group7: Diabetic + Glibenclamide (600μg/kg BW). The animals were maintained for the period of 45 days. 1 mL of Bacoside-A and Glibenclamide dissolved in water were administered by intragastric intubation for 45 days of the experimental period. Initial and final body weight of the animals were noted everyday. Daily intake of food and water were monitored for 45 days of all experimental animals. Fixed quantity of standard pellet diet and water were provided for each rat and replenished the next day.

2.5. Sample collection

At the end of experimental period rats were fasted overnight and sacrificed by cervical dislocation. Blood was collected through jugular vein and centrifuged at 1500 g for 10 min for the analysis of hepatic markers. Liver tissue was excised instantaneously from the experimental rats and washed in ice-cold isotonic saline and blotted with a filter paper. A slice of the tissue was weighed, homogenised in 0.1 M Tris–HCl buffer (pH 7.4) and centrifuged at 3000 g for 10 min at 0 °C in cold centrifuge. Homogenised tissue was used for carbohydrate metabolic enzyme and glycogen metabolic enzymes estimations. Fresh hepatic tissue was used for the analysis of hepatic glycogen content. For histological study, hepatic tissue was collected in 10% formalin solution and immediately processed by the paraffin technique.

2.6. Biochemical estimations

Plasma glucose levels were estimated by a commercial kit (Sigma Diagnostics Pvt. Ltd., Baroda, India) by the method of Trinder (1969). Plasma insulin was assayed by ELISA kit (Boeheringer–Manneheim Kit, Mannheim, Germany). Total hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) were estimated in the whole blood by diagnostic kits (Agappe Diagnostic Pvt. Ltd., India). The carbohydrate metabolic enzymes were determined by the levels of hepatic hexokinase activity (Brandstrup et al., 1957), Glucose-6- phosphate dehydrogenase (G6PD) (Ells and Kirkman, 1961), fructose-1,6-bisphosphatase (Gancedo and Gancedo, 1971) and glucose-6-phosphatase activity (Koide and Oda, 1959) in liver of the experimental animals. Glycogen metabolic enzymes were assessed by the levels of glycogen content (Morales et al., 1973) and Glycogen synthase (Golden et al., 1977) and glycogen phosphorylase activity (Shull et al., 1956) respectively in liver of the experimental rats. Hepatic markers such as aspartate transaminase (AST) and alanine transaminase (ALT) (Reitman and Frankel, 1957) were assayed.

Table 1
Effect of bacoside-A on the body weight, food and water intake in normal control and STZ induced diabetic rats.

Groups	Body weight (g)		Food intake (g/rat/day)	Water intake (mL/rat/day)
	Initial (0th day)	Final (45th day)		
Normal Control	189.14 ± 4.01	211.54 ± 5.93^{a}	16.32 ± 1.14^{a}	68.75 ± 3.91^{a}
Normal + Bacoside-A (20 mg/kg BW)	181.51 ± 3.93	210.78 ± 6.71^{b}	17.08 ± 1.19^{a}	69.17 ± 4.15^{a}
Diabetic Control	189.65 ± 3.24	139.85 ± 4.18^{c}	67.18 ± 4.38^{b}	156.39 ± 11.76^{b}
Diabetic + Bacoside-A (5 mg/kg BW)	180.74 ± 4.76	189.63 ± 5.02^{d}	41.71 ± 3.01^{c}	111.36 ± 8.15^{c}
Diabetic + Bacoside-A (10 mg/kg BW)	183.46 ± 5.91	199.17 ± 6.14^{e}	29.36 ± 1.97^{d}	93.54 ± 7.03^{d}
Diabetic + Bacoside-A (20 mg/kg BW)	187.03 ± 4.95	191.26 ± 5.84^{d}	36.15 ± 2.18^{e}	104.86 ± 7.69^{e}
Diabetic + Glibenclamide (600μg/kg BW)	182.12 ± 4.86	204.37 ± 5.17^{e}	25.91 ± 2.08^{d}	84.12 ± 6.54^{d}

Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Table 2
Effect of bacoside-A on the fasting glucose and insulin in the plasma of normal control and STZ induced diabetic rats.

Groups	Fasting glucose (mg/dL)	Insulin (μU/mL)
Normal Control	90.41 ± 5.90^{af}	15.25 ± 1.26^{ab}
Normal + Bacoside-A (20 mg/kg BW)	88.76 ± 5.46^{a}	16.18 ± 1.31^{b}
Diabetic Control	279.75 ± 11.22^{b}	7.03 ± 0.54^{c}
Diabetic + Bacoside-A (5 mg/kg BW)	165.13 ± 13.62^{c}	8.92 ± 0.79^{d}
Diabetic + Bacoside-A (10 mg/kg BW)	123.47 ± 11.74^{d}	13.48 ± 1.20^{e}
Diabetic + Bacoside-A (20 mg/kg BW)	145.28 ± 12.22^{d}	11.13 ± 1.41^{f}
Diabetic + Glibenclamide (600μg/kg BW)	101.78 ± 7.02^{e}	14.58 ± 1.47^{ae}

Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

2.7. Histopathological study

Liver tissues were collected from experimental animals and were fixed in 10% neutral buffered formalin solution, dehydrated in ethanol and embedded in paraffin. Sections of 5 μm thickness were arranged using a rotary microtome and stained by means of hematoxylin and eosin dye and mounted in a neutral deparaffinated xylene medium for microscopic observation.

2.8. Statistical analysis

Data represented as means ± standard deviation (SD) and subjected to statistical significance were calculated by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using Statistical Package for the Social Sciences (SPSS) Version 20.0 (SPSS, Cary, NC, USA). Values of $p < 0.05$ were considered as statistically significant.

3. Results and discussion

The chronic hyperglycemia is the upshot of perturbation in blood glucose homeostasis. T2DM is a global epidemic characterized by diminished glucose homeostasis due to insulin deficiency and tissue resistance to insulin invigorated glucose uptake and utilization. Ergo, upsurge attention of researchers towards the management of T2DM and its complications due to its unabated incidence.

Table 1 delineates the measurements of the initial, final body weight, food and water intake of all experimental animals. Body weight significantly ($p < 0.05$) diminished whereas food and water intake significantly increased in diabetic animals compared to normal control rats. The prevailing reason may be due to lacking insulin secretion in that way it elevates glycogenolysis ultimately increased muscle wasting in diabetes, protein wasting because of insufficient availability of carbohydrate for utilization as an energy source (Musabayane et al., 2005). Bacoside-A and glibenclamide administered diabetic rats showed notable progress in body weight, diminution in food and water intake. Similar findings of improvement in body weight amongst diabetic animals are reliable with treatment of triterpenoid which is already reported to have potential anti-diabetic effects (Mandal et al., 2015).

A siginificant increase in the levels of fasting plasma glucose while a significant ($p < 0.05$) decrease in the level of plasma insulin in diabetic rats than the normal control rats. Daily administration of bacoside-A (5, 10 and 20 mg/kg BW) for 45 days to diabetic rats showed a significant ($p < 0.05$) reduction in fasting plasma glucose and increased in plasma insulin levels when compared with diabetic rats (Table 2). STZ causes partial destruction of ß-cells leads to the deficiency in insulin secretion, as significant reduction of insulin levels in Type 2 diabetic (T2D) rats which closely resemble the metabolic disturbances characterization in T2DM patients as linked to hyperglycemic animals (Ayesha et al., 2017).

Table 3
Effect of bacoside-A on the levels of Hb and HbA1c in plasma of normal control and STZ-diabetic rats.

Groups	Hemoglobin (g/dL)	Glycosylated hemoglobin (mg/g of Hb)
Normal Control	14.51 ± 1.26^{a}	0.40 ± 0.02^{a}
Normal + Bacoside-A (20 mg/kg BW)	$13.87 \pm 1.24^{a,b}$	0.37 ± 0.02^{a}
Diabetic Control	7.05 ± 0.44^{c}	1.03 ± 0.06^{b}
Diabetic + Bacoside-A (5 mg/kg BW)	10.01 ± 0.90^{d}	0.77 ± 0.05^{c}
Diabetic + Bacoside-A (10 mg/kg BW)	12.53 ± 0.84^{e}	0.51 ± 0.04^{d}
Diabetic + Bacoside-A (20 mg/kg BW)	10.92 ± 0.92^{d}	0.66 ± 0.03^{e}
Diabetic + Glibenclamide (600μg/kg BW)	$13.16 \pm 1.07^{a,e}$	0.45 ± 0.02^{f}

Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Table 4
Effect of bacoside-A on the activity of hexokinase and glucose-6-phosphate dehydrogenase in the liver of normal control and diabetic rats.

Groups	Hexokinase (U*/h/mg protein)	Glucose-6-phosphate dehydrogenase ($U^{\neq}$/mg protein)
Normal Control	0.31 ± 0.01^{a}	4.63 ± 0.41^{a}
Normal + Bacoside-A (20 mg/kg BW)	0.29 ± 0.01^{b}	5.04 ± 0.38^{b}
Diabetic Control	0.14 ± 0.01^{c}	2.28 ± 0.17^{c}
Diabetic + Bacoside-A (5 mg/kg BW)	0.17 ± 0.02^{d}	3.02 ± 0.21^{d}
Diabetic + Bacoside-A (10 mg/K BW)	0.23 ± 0.01^{e}	3.90 ± 0.23^{e}
Diabetic + Bacoside-A (20 mg/K BW)	0.20 ± 0.01^{f}	3.12 ± 0.27^{d}
Diabetic + Glibenclamide (600μg/KgBW)	0.27 ± 0.02^{g}	4.17 ± 0.34^{e}

U* - μmol of glucose phosphorylated per hour.
$U^{\#}$- nmol of NADPH formed per minute.
Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Neverthless, the plasma glucose level of normal rats remains to be unchanged while upon treatment with bacoside-A, clearly exhibits the normoglycemic impact of the compound. Moreover, the diminution in the fasting plasma glucose and elevation of plasma insulin levels of the bacoside-A treated diabetic rats suggests that it might the stimulation of a regeneration and revitalization of the remnant ß-cells by blocking ATP sensitive K+ channel. Similar action of glibenclamide on ß-cells secreting insulin promote glucose uptake into the peripheral tissues (Bolkent et al., 2000). However, previous report showed that triterpenoids serves as the regulator of plasma glucose level via inhibiting ATP sensitive K^{+} Channels in STZ-diabetic rats (Jang et al., 2010). Therefore, bacoside-A serve as a efficient insulin secretagogue property by reducing hyperglycemia. Treatment of bacoside-A revealed dose dependent effects on plasma glucose and plasma insulin levels throughout the experimental period, portraying its potent antihyperglycemic effect. Finally, 10 mg/kg BW per day was more prominently in reducing the plasma glucose tends to normalcy and it was fixed as the effective dose for further investigation.

Glycosylated haemoglobin (HbA1c) considered as a diabetic marker for assessing the degree of protein glycation in diabetes and its complications. HbA1c is non enzymatic glycated product from the glycosylation of the N-terminal valine residues of ß chain of haemoglobin by

Table 5
Effect of bacoside-A on the activity of gluconeogenic enzymes in the liver of normal control and diabetic rats.

Groups	Glucose 6-phosphatase (U[@]/min/mg protein)	Fructose 1,6 -bisphosphatase (U[$]/h/mg protein)
Normal Control	0.20 ± 0.01^{a}	0.34 ± 0.02^{a}
Normal + Bacoside-A (20 mg/kg BW)	$0.19 \pm 0.01^{a,b}$	0.30 ± 0.01^{b}
Diabetic Control	0.40 ± 0.03^{c}	0.73 ± 0.03^{b}
Diabetic + Bacoside-A (5 mg/kg BW)	0.32 ± 0.02^{d}	0.59 ± 0.04^{d}
Diabetic + Bacoside-A (10 mg/kg BW)	0.26 ± 0.01^{e}	0.47 ± 0.02^{e}
Diabetic + Bacoside-A (20 mg/kg BW)	0.30 ± 0.02^{d}	0.56 ± 0.02^{d}
Diabetic + Glibenclamide (600μg/kg BW)	0.22 ± 0.01^{b}	0.40 ± 0.02^{f}

U@-μmol of Pi liberated per min.
U$- μmol of Pi liberated per h.
Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Table 6
Effect of bacoside-A on the glycogen content and activity of glycogen metabolic enzymes in the liver of normal control and diabetic rats.

Groups	Glycogen (mg/100 g tissue)	Glycogen synthase (U*/h/mg protein)	Glycogen phosphorylase (U[#]/h/mg protein)
Normal Control	42.17 ± 3.55^{a}	831.74 ± 23.80^{a}	625.41 ± 16.71^{a}
Normal + Bacoside-A (20 mg/kg BW)	46.89 ± 3.42^{b}	840.21 ± 21.81^{a}	629.32 ± 15.92^{a}
Diabetic Control	22.11 ± 1.52^{c}	515.92 ± 28.20^{b}	893.76 ± 22.86^{b}
Diabetic + Bacoside-A (5 mg/kg BW)	26.37 ± 1.56^{d}	696.17 ± 21.08^{c}	815.12 ± 16.98^{c}
Diabetic + Bacoside-A (10 mg/kg BW)	32.80 ± 1.79^{e}	773.68 ± 23.03^{d}	764.31 ± 16.15^{d}
Diabetic + Bacoside-A (20 mg/kg BW)	28.46 ± 1.63^{d}	701.69 ± 21.14^{c}	801.5 ± 16.53^{c}
Diabetic + Glibenclamide (600μg/kg BW)	37.28 ± 2.06^{f}	803.52 ± 20.08^{e}	747.15 ± 18.18^{d}

U*- mmoles of UDP formed/h/mg protein.
U[#]- mmoles Pi liberated/h/mg protein.
Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

circulating glucose. The level of Hb was significantly declined whereas the level of HbA1c was significantly ($p < 0.05$) elevated in diabetic rats (Table 3) however when treated with bacoside-A and glibenclamide, showed these values were brought toward near normalcy. In hyperglycemia condition, non enzymatic glycation reaction is increased due to supra physiological glucose. HbA1c was ascertaining to significantly enhance in diabetic persons and the extent of increase in HbA1c is directly proportionate to the fasting blood glucose level (Qiao et al., 2019). Declined in the level of total haemoglobin (Hb) in diabetic rats is primarily due to the augmented formation of HbA1c. Administration of bacoside-A markedly reduced the elevation of HbA1c thus improving the total Hb levels in diabetic rodents as compared with the standard reference drug. This could be due to the result in improving glycemic control exerted by bacoside-A. Ghosh et al. (2011) reported that bacosine exhibits a glycemic control which were correlated with our results.

Impact of bacoside-A on carbohydrate metabolic enzymes of normal and experimental rats were demonstrated in Tables 4 and 5. The diabetic group showed remarkably reduction in activity of hexokinase, glucose-6-phosphate dehydrogenase whereas significant increase in activity of glucose-6-phosphatase and fructose-1,6-bisphosphatase. Administration of bacoside-A and glibenclamide brought back these parameters to near normalcy. The activity of hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase were considerably ($p < 0.05$) elevated in T2D rats compared to normal control rats. However, administration with bacoside-A or glibenclamide to T2D rats significantly ($p < 0.05$) reduced the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase as compared to diabetic rats.

Liver serves as a metabolic hub and most of its metabolic activity is firmly regulated by insulin, glucagon and growth hormone. Normoglycemia is tightly controlled by the integration and associated regulation of several metabolic pathways including gluconeogensis and glycolysis. Hexokinase plays a prime role in blood glucose homeostasis and it functions as the glucose sensor in the liver allowing hepatocytes to crucial trapping of glucose in response to glycemic fluctuations in its active state. Hexokinase is catalyzing the formation of glucose 6-phosphate (G6P) by phosphorylation reaction of glucose. G6P is a downstream metabolite which acts as an active molecule in regulating glycogen synthesis pentose phosphate pathway and glycolysis. In this study, experimental T2D rats exhibited diminished activity of hexokinase which sequentially decrease the glucose utilization in hepatocytes. Subsequent administration of bacoside-A and glibenclamide depicted significant elevated activity of hexokinase in diabetic rats and therefore it facilitate glucose utilization through glycolysis and pentose phosphate pathway. In previous study (Ramachandran and Saravanan, 2013) described that asiatic acid has improved glycolytic enzymes.

G6PD is considered as the archetypical X-linked "housekeeping" enzyme, which plays the crucial role in controlling glucose flux through the pentose phosphate pathway. G6PD plays a dynamic role in glucotoxicity which specifically catalyzes the initial step from glucose-6-phosphate to 6-phosphogluconate in the pathway finally end products including pentose phosphates and reducing equivalent, reduced nicotinamide adenine dinucleotide phosphate (NADPH). Depletion in the activity of G6PD progressively slows down the pentose phosphate pathway in diabetic conditions, which influences NADPH concentration involved in reductive biosynthesis and stability of the redox state of the cell associated with oxidative stress, leading to diabetic complications. In this study, elevated activity of cytosolic enzyme G6PD in bacoside-A treated diabetic rats by enhancing the lipogenesis through utilization of NADPH and ultimately attenuating hyperglycemia. This is symmetry with the contribution of D-limonene, a monoterpene in modulating G6PD in STZ induced diabetic rats (Murali and Saravanan, 2012).

Augmented endogenous glucose production is considered as a hallmark of T2DM. Nevertheless the distinguishable criterion of diabetes is hyperglycaemia were availability of glucose to the tissues is restricted. Glucose 6-phosphatase, a key enzyme involved in the gluconeogenesis and glycogenolysis that catalyzing both of the terminal step in the metabolic processes i.e., the dephosphorylation of G6P to glucose. Glucose 6-phosphatase activity ruins glucose utilization in the liver, whilst concurrently increasing hepatic glucose production. Another target hepatic gluconeogenic enzyme is fructose 1,6-bisphosphatase. Fructose 1,6-bisphosphatase catalyzes the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate, a penultimate step in gluconeogenesis pathway. Glucose 6-Phosphatase and fructose 1,6-bisphosphatase activities remain outstanding during the diabetic condition (Dhamodaran and Natarajan, 2012). Treatment with bacoside-A assisted in gaining metabolic regulator over these enzymes for the purpose to normalize the glucose concentration in the blood circulation. The gluconeogenic enzyme activities were released remarkably, hence restoring a glycaemic profile in bacoside-A treated T2D rats (Raju et al., 2013). These outcomes implied that the beneficial efficacy of bacoside-A proves not alone in reducing hyperglycaemia and management of related complications which may affects the liver such as fatty liver disease.

The normoglycemic hormone, insulin impact on glucose reserve acts a pivotal role in glycogen metabolism. In physiological condition, liver serves as an imperative role in buffering the postprandial hyperglycemia ultimately involved in glycogenesis by utilizing excess glucose. In long

Table 7
Effect of bacoside-A on the activity of hepatic marker enzymes in the normal control and diabetic rats.

Groups	AST (IU/L)	ALT (IU/L)
Normal Control	74.61 ± 4.51^{ab}	26.71 ± 2.19^{a}
Normal + Bacoside-A (10 mg/kg BW)	70.23 ± 4.22^{b}	24.67 ± 1.95^{a}
Diabetic Control	116.52 ± 8.98^{c}	65.83 ± 5.77^{b}
Diabetic + Bacoside-A (10 mg/kg BW)	85.17 ± 5.93^{d}	34.1 ± 2.68^{c}
Diabetic + Glibenclamide (600µg/kg BW)	80.5 ± 4.56^{ad}	30.94 ± 2.89^{c}

Values are given as means ± S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

term diabetes, there is an impairment in synthesis of liver glycogen owing to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis (Murali and Saravanan, 2012). In consequence the determination of glycogen, the primary intracellular depository of glucose in liver and glycogen metabolic enzymes can be considered as a preeminent indicator of T2DM. Disturbances in glycogen metabolic enzymes like glycogen synthase and glycogen phosphorylase which involved in synthesis of glycogen and breakdown of glycogen respectively in diabetes. The hepatic glycogen content and its metabolizing enzymes illustrated in Table 6. Liver glycogen content and glycogen synthase activity were drastically decreased whereas glycogen phosphorylase activity was exaggerated in diabetic control compared to normal control (Table 6). Reverted these parameters towards near normalcy in bacoside-A treated STZ rats. In our study, the diabetic rats illustrated diminished hepatic glycogen content and glycogen synthase activities contemporarily elevated glycogen phosphorylase activities. Administration of bacoside-A in T2D rats exhibited the reversal of forementioned parameters this is probably due to enhanced insulin action. The insulin secretagogue activity of bacoside-A was further substantiated through its amended hepatic glycogen content and its enzymes in the hepatic tissue of treated diabetic rats. Naresh et al., 2012 reported that Irdiod glucoside, a monoterpenoid possessing similar effect in glycogen and its metabolizing enzymes.

Conventionally, STZ damages pancreatic ß-cell and it deteriorates greatly towards hepatocytes owing to metabolic hub nature of liver. Considering this point, hepatic markers including AST and ALT in serum of experimental rats are analysed. Serum AST and ALT activities in experimental rodents are represented in Table 7. The activity of AST and ALT were dramatically raised in diabetic group. A notable increased in hepatic markers enzymes activities in bacoside-A and glibenclamide treated diabetic rats. Hyperglycemia is usually accompanied with progressively escalated in the hepatic marker enzymes activity (Haroon and Murali, 2016). Due to hepatic damage, increase in activity of hepatic enzymes in the serum of diabetic rats may be due to the outflowing of these specific enzymes from the cytosol of the liver into blood stream (Mori et al., 2003). However the administration of bacoside-A significantly rendered the activities of AST and ALT in diabetic rats seems to possess hepatoprotective nature of the bacoside-A. We studied hepatic markers and its histopathology in experimental design 2 based on dose

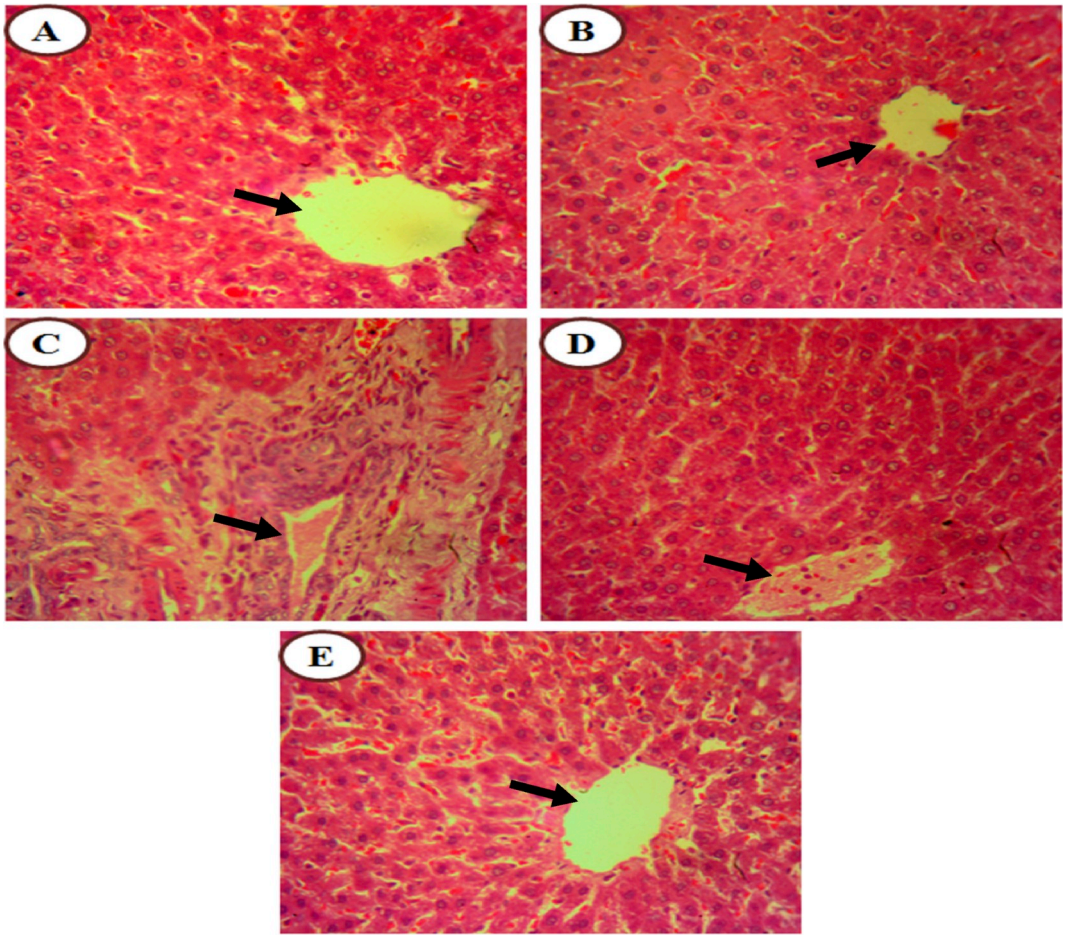

Fig. 1. Histopathology section of liver of normal control and experimental rats (H and E 40 ×). Normal control (A) Normal hepatocytes with a central vein, Normal + Bacoside-A (10 mg/kg BW) (B) Intact architecture and normal hepatocytes, Diabetic control rats (C) Hepatocytes degeneration with foamy macrophage and sinusoid dilation, Diabetic + Bacoside-A (10 mg/kg BW) (D) Normal cellular architecture with distinct hepatic cells sinusoidal spaces, Diabetic + Glibenclamide (600µg/kg BW) (E) Near normal histology.

fixation study of experimental design1 and followed by administration of bacoside-A, a dose of 10 mg/kg BW was more pronounced effect than other doses (5,20 mg/kg BW) in both groups [Normal + Bacoside-A (10 mg/kg BW) and Diabetic + Bacoside-A (10 mg/kg BW)] compared than normal control rats.

Diabetic liver showed that a histopathological change includes fatty changes in hepatocytes, congestion of sinusoids and focal necrosis. However, T2D rats treated with bacoside-A showed normal hepatocytes around the central vein with a bridged necrosis in the liver (Fig. 1). Based on histopathology results, normal hepatocytes maintaining normoglycemia by means of faster the rate of glucose transport through GLUT2 (Thorens,2015) into hepatocytes in which regulates carbohydrate metabolic enzymes and liver function enzymes in order to reversed in diabetic condition.

4. Conclusion

Noteworthy to conclude from fore-mentioned findings, bacoside-A (10 mg/kg BW) administration to T2D rats revealed reinstatement of plasma glucose, body weight, plasma insulin, hepatic carbohydrate metabolic regulatory enzymes in glucose homeostasis. Histopathological investigation of liver section was supported the analytic results of biomacromolecules. Therefore, bacoside-A exerts antidiabetic properties and hence it might be a possible candidate for an anti-diabetic drug. Further clinical studies are recommended in the future determined effort to confirm the experimental studies.

Conflicts of interest

Authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bcab.2019.101331.

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