Medical and Clinical Archives

Research Article ISSN: 2515-1053

Effect of flower and root of Aerva lanata (L.) on enzymes of carbohydrate metabolism in alloxan induced diabetic rats

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Abstract

Background: Diabetes mellitus is the most common endocrine disorders that impair glucose homeostasis resulting in hyperglycemia and glycosuria.

Methods: The present study was to evaluate the effect of flower and root of Aerva lanata (L.) on the activities of enzymes of carbohydrate metabolism in alloxan (150 mg/kg bw) induced diabetic rats. Oral administration of aqueous extracts of flower and root of *A. lanata* (200 mg/kg bw) to diabetic rats was done daily for 45 days.

Results: This study showed that decreased levels of hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase and glycogen synthase and increased levels of lactate dehydrogenase and glycogen phosphorylase activities in liver of diabetic rats. The elevated levels of enzyme activities such as hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase and lactate dehydrogenase were observed in kidney of diabetic rats. The plant extracts treated diabetic rats showed that the above-mentioned enzymes activities were near normal as like as normal control rats. In liver and kidney, the gluconenogenic key enzymes like glucose-6-phosphatase and fructose-1, 6-bisphosphatase were increased in diabetic rats when compared to normal control rats. After oral administration of flower and root extracts of *A. lanata* treated to diabetic rats showed that decreased levels of above mentioned enymes activities.

Conclusion: The results were compared and observed more or less similar with standard drug glibenclamide (1mg/ kg bw) treated rats. So, the present study confirmed that the antidiabetic effect of flower and root of *A. lanata* on alloxan induced diabetic rats by maintaining the normal levels of carbohydrate metabolizing enzymes.

Introduction

Carbohydrates play a several crucial roles in the metabolic processes of living organisms. They serve as energy sources and as structural elements in living cells. The main role of carbohydrates is involved in the energy production process, because the glucose is a prominent energy source in almost all living cells and tissues and the major emphasis is placed on its synthesis, degradation, and storage. Living cells are in a state of ceaseless activity to maintain its life and each cell depends on highly coordinated several biochemical reactions. Carbohydrates are an important source of energy compound than protein. The energy-generating pathways of carbohydrate metabolism are glycolysis and tricarboxylic acid cycle. It is an ancient pathway found in almost all organisms, a small amount of energy is captured in the process of glucose molecule is converted to two molecules of pyruvate. Glycogen, a storage form of glucose in vertebrates is synthesized by glycogenesis when glucose levels are high and degraded by glycogenolysis when glucose is in short supply. Glucose can also be synthesized from non-carbohydrate precursors by reactions referred to as gluconeogenesis. The pentose phosphate pathway enables cells to convert glucose-6-phosphate, a derivative of glucose, to ribose- 5-phosphate (the sugar used to synthesize nucleotides and nucleic acids) and other types of monosaccharides. NADPH, an important cellular reducing agent, is also produced by pentose phosphate pathway [1].

Carbohydrate metabolism is focused on the synthesis and usage of glucose, a major fuel for most organisms. In vertebrates, glucose is transported throughout the body through blood. If cellular energy reserves are low, glucose is degraded by the glycolytic pathway. Glucose molecules not required for immediate energy production are stored as glycogen in liver and muscle. The energy requirements of many tissues (e.g., brain, red blood cells, and exercising skeletal muscle cells) depend on an uninterrupted flow of glucose. Depending on a cell's metabolic requirements, glucose can also be used to synthesize, for example, other monosaccharides, fatty acids, and certain amino acids [1]. Insulin is an important hormone in carbohydrate metabolism. Due to insulin secretion is insufficient or impaired that leads to diabetes mellitus.

Diabetes mellitus is a group of heterogenous, hormonal and metabolic disorders characterized by hyperglycemia and glucosuria with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion and or insulin action [2]. Diabetes mellitus, a chronic disorder is associated with long-term complications, including retinopathy (eye), nephropathy (kidney) and angiopathy (heart). Diabetes mellitus is considered to be a major risk factor for cardiovascular disorders namely ischemic heart disease, cerebral stokes and peripheral artery disease leading to increased mortality of diabetes [3]. The prevalence of diabetes is increasing rapidly worldwide and the World Health Organization (2003) has

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Key words: *aerva lanata, liver, kidney, metabolic enzymes*

Received: September 03, 2018; **Accepted:** September 14, 2018; **Published:** September 17, 2018

predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 370 million. Experts project that the incidence of diabetes is set to soar by (64%) by 2025, meaning that a staggering 53.1 million citizens will be affected by the disease [4]. The estimated worldwide prevalence of diabetes among adults in 2010 was 285 million (6.4%) and this value is predicted to rise to around 439 million (7.7%) by 2030 [5].

Many drugs are available for the treatment of diabetes, but their long-term use may cause adverse side effects and hence, the increased search for natural remedies to effective treatment of diabetes exists [6]. Plants have always been a very good source of drugs and many of the presently available drugs are directly or indirectly made from them [7]. Plants used for medicinal purposes are frequently considered to be less toxic and induces fewer side effects than synthetic ones. Plants are most often implicated as having anti-diabetic effects contain glycosides, alkaloids, terpenoids, flavonoids and carotenoids [8]. Antihyperglycaemic effects of these plants are due to their capability to improve the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or the facilitation of metabolites in insulin dependent processes [9]. More than 800 plant species have been reported to be possessing hypoglycemic activity in available literatures [10]. In recent times, focus on plant research has increased throughout the world and the immense potential of medicinal plants used in various traditional systems has been established scientifically. Screening of plants with ethno medical use is being lived to increase the odds in discovering new medicines [11]. In ancient times, medicinal plants and herbs were used as remedy for serious health complications. Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world. Many indigenous Indian medicinal plants have been found useful for managing diabetes [12,13].

Aerva lanata is an important medicinal plant of Amranthaceae and known as polpala. It is a prostrate to decumbent sometimes erect herb, found throughout tropical India as a common weed in field and wasteland. The plant is used for curing diabetes, anthelmintic, demulcent and it is helpful in lithiasis, cough, sore throat and wounds. The plant has been used as diuretic, antidiabetic, expectorant and hepatoprotective in traditional system of medicine [14]. The plant *A. lanata* possess antimicrobial and cytotoxicity [15], diuretic [16], urolithiasis [17] and anti-inflammatory [18], antibacterial [19] activities. It has been reported that Canthin-6-one and β-carboline alkaloids were isolated from leaves of *A. lanata* [20]. The alcoholic extract of shoots of *A. lanata* has shown significant antidiabetic activity. There is no detailed study on antidiabetic activity of flower and root of *A. lanata*. So, the present study was aimed to determine the effect of aqueous extracts of flower and root of *A. lanata* on carbohydrate metabolic enzymes activities in alloxan induced diabetic rats.

Materials and Methods

Collection and preparation of plant material: The medicinal plant Aerva lanata was collected from in and around Mayiladuthurai at Nagapattinum District, Tamilnadu, India. The plant was identified and authenticated by Dr. S. John Britto, Director, Rapinat Herbarium and Centre for Molecular Systematics, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. The flower and root of *A. lanata* were separated and washed thoroughly in running tap water to remove soil particles and adhered debris and then finally washed with sterile distilled water. The flower and root of *A. lanata* were dried under shade and ground well into powder. The powdered materials were stored in air tight containers untill the time of use.

Preparation of aqueous extracts: 30 g powder of flower and root were soaked separately in distilled water for 12 to 16 hours and boiled and then it was filtered through muslin cloth and then Whatmann no. 1 filter paper. The aqueous extracts were concentrated and made the final volume to one-fifth of the original volume [21]. The paste form of extracts was stored in an air tight container at 4 °C.

Experimental animals: Albino rats (Wistar) weight ranges between 150-200 g of both sexes were used for this study. They were housed in polypropylene cages under standard laboratory conditions (12-h light / 12-h dark cycle, 21 ± 2 °C, and relative humidity 55 %). The animals were given standard rodent pellets and water ad libitum. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. Ethical clearance for handling animals for this study was obtained from Institutional Animal Ethical Committee (IAEC) of SASTRA University, Thanjavur, Tamilnadu India. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (Approval no: 211/ SASTRA /IAEC/RPP).

Induction of diabetes mellitus: Diabetes was induced by the single intraperitoneal injection of alloxan at the dose of 150 mg/kg b.w. The alloxan was dissolved in distilled water, and then used to induce diabetes mellitus. Fasting blood glucose of the animals was measured after 14 days of alloxan injection using glucometer. Rats with fasting blood glucose above 200 mg/dl were considered as diabetic rats.

Drug administration: After 14 days of alloxan induction, the aqueous extract of flower and root of A. lanata were administered orally through intragastric tube at the dose of 200 mg kg-1 body weight.

Group I: Normal control – received only distilled water during the experimental period.

Group II: Diabetic control.

Group III: Diabetic rats were daily treated with *A. lanata* flower extract (200 mg /kg body weight) dissolved in distilled water by orally through intragastric tube for 45 days.

Group IV: Diabetic rats were daily treated with *A. lanata* root extract (200 mg/kg body weight) dissolved in distilled water by orally through intragastric tube for 45 days.

Group V: Diabetic rats were daily treated with standard drug glibenclamide (1 mg/kg body weight) dissolved in distilled water by orally through intragastric tube for 45 days.

Collection of blood sample and tissues: After the experimental period, the animals were kept fasted overnight and sacrificed by cervical decapitation under mild anesthesia. Blood was collected on decapitation and serum was separated by centrifugation at 2500 rpm for 15 min. The collected serum was used for the analysis of the activities of ALT, AST and γGT. Liver and kidney were dissected out immediately rinsed with ice cold saline and used for the further study like analysis of metabolic enzymes activities.

Biochemical studies

The collected samples like serum and tissues were subjected to biochemical analysis. Blood glucose was analysis by Folins Wu method [22], AST and ALT were estimated by the method of Reitman et al. [23], gamma glutamyl transpeptidase was assayed by the method of Szasz et al. [24] and hexokinase was assayed by the method of Brandstrup et al. [25]. Pyruvate kinase was estimated by the method of Pogson et al. [26] and lactate dehydrogenase was assayed by the method of King [27]. Glucose -6-phosphate dehydrogenase was measured the by the

method of Gancedo and Gancedo [28], glucose -6-phosphatase was assayed by the method of Koide et al. [29], fructose-1-6-diphosphatase was estimated by the method of Gancedo and Gancedo [28], glycogen content was assayed by the method of Carroll et al. [30], glycogen synthetase and glycogen phosphorylase were assayed by the method of Leloir and Goldemberg [31] and Carnblath et al. [32] respectively.

Statistical analysis

The results of the present study were subjected to statistical analysis and all the results were expressed as means ± SE. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS (statistical package for social sciences) Ver. 11 and the individual comparison were obtained by Duncan's Multiple Range Test (DMRT). The values were considered statistically significant at 5% level ($p \leq 0.05$). Values with identical letter are not significantly different according to DMRT at 5% level.

Results

The level of blood glucose and the activities of enzymes like aspartate transaminase (AST), alanine transaminase (ALT) and gamma glutamyl transpeptidase (γGT) were analyzed in normal and experimental rats the results were represented in (Table 1). The increased levels of activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (γGT) were observed in serum of diabetic rats when compared with normal control rats. After administration of aqueous extracts of flower and root of *A. lanata* to diabetic rats showed significantly decreased levels of AST, ALT and γGT, which were more or less similar to standard drug glibenclamide (1mg/ kg bw) treated rats.

The effect of flower and root extracts of *A. lanata* on hexokinase, pyruvate kinase, lactate dehydrogenase, glucose -6-phosphate dehydrogenase, glucose-6- phosphatase and fructose-1,6-bisphosphatase in alloxan induced diabetic rats was studied and the results were presented in (Table 2). The activities of hexokinase, pyruvate kinase and glucose-6-phophate dehydrogenase were decreased in liver of diabetic rats, but the activity of lactate dehydrogenase was significantly increased. After the plant extracts were administered to diabetic rats significantly increased levels of enzymes activities of hexokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase was observed. The administration of root extract of *A. lanata* showed predominant recovery than flower. In the present study, the glucose-6-phosphatase and fructose 1, 6- bisphosphatase activities were elevated in liver of alloxan induced diabetic rats. After 45 days, aqueous extracts of flower and root of *A. lanata* (200 mg/kg b.w.) treated diabetic rats showed that decreased levels of gluconeogenic key enzymes activities in liver. The root of *A. lanata* showed higher activity than flower extract.

The effect of flower and root extracts of *A. lanata* on carbohydrate metabolic enzymes like hexokinase, pyruvate kinase, glucose-6 phosphate dehydrogenase, lactate dehydrogenase, glucose-6-

Table 1. Effect of flower and root extracts of *A. lanata* on glucose, AST, ALT and γGT. (Values are expressed as means ± S.E. of six samples from each group,Values are not sharing a common superscript differ significantly at 5% level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT)).

Groups	Blood sugar (mg/dl)	AST (IU/L)	ALT (IU/L)	γGT (IU/L)
Group I Normal control	92.74±3.64^{a}	29.81±1.17^{a}	77.64±3.05^{b}	18.43±0.72^{a}
Group II Diabetic control	286.72± 11.26^{d}	59.47±2.33^{d}	118.28±4.64^{d}	30.66±1.20^{c}
Group III Diabetic + Flower extract (200mg/ kg bw)	143.34±5.63^{c}	45.90±1.80^{c}	102.22±4.01^{c}	25.62±1.00^{b9}
Group IV Diabetic + Root extract (200mg/kg bw)	119.44±4.69^{b}	37.01±1.45^{b}	84.72±3.32^{b}	20.49±0.80^{a}
Group V Diabetic + Glibenclamide (1mg/kg bw)	101.47±3.98^{a,b}	32.69±1.28^{a,b}	80.46±0.76^{a}	19.4±0.76^{a}

Table 2. Effect of flower and root extracts of *A. lanata* on metabolic enzymes like hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and fructose-1,6-bisphosphatase in liver. (Values are expressed as means ± S.E. of six samples from each group, Values are not sharing a common superscript differ significantly at 5% level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT)).

Groups	Hexokinase (µmoles of glucose -6-phosphate formed / hr/mg of protein)	Pyruvate kinase (µmoles of pyruvate formed /min/mg of protein)	Lactate dehydrogenase (µmoles of pyruvate formed/hr/mg of protein)	Glucose -6-phosphate dehydrogenase (U/min/mg of protein)	Glucose-6-phosphatase (µmoles of phosphate liberated/ hr/mg of protein)	Fructose-1,6-bis phosphatase (µmoles of phosphate liberated/hr/mg of protein)
Group I Normal control	260.51±10.23^{c}	10.65±0.41^{d}	239±9.39^{a}	2.39±0.09^{d}	1034.52±40.65^{a}	498.89±19.60^{a}
Group II Diabetic control	128.84±5.06^{a}	4.65±0.18^{a}	473.49±18.60^{c}	0.96±0.03^{a}	1921.79±75.52^{c}	816.12±32.07^{c}
Group III Diabetic + Flower extract (200mg/kg bw)	204.11±8.02^{b}	6.36±0.25^{b}	306.12±12.02^{b}	1.47±0.05^{b}	1229.21±48.30^{b}	715.14±28.10^{b}
Group IV Diabetic + Root extract (200mg/kg bw)	253.2±9.95^{c}	8.81±0.34^{c}	265.31±10.42^{a}	2.25±0.09^{c,d}	1048.56±41.20a	511.23±20.09^{a}
Group V Diabetic + Glibenclamide (1mg/kg bw)	254.22±9.99^{c}	9.39±0.36^{c}	275.50±10.82^{a,b}	2.14±0.08^{c}	1123.33±44.14^{a,b}	556.11±21.85^{a}

phosphatase and fructose 1, 6- bisphosphatase in alloxan induced diabetic rats were studied and the results were given in (Table 3). The present study showed that the increased levels of hexokinase, pyruvate kinase, glucose-6 phosphate dehydrogenase, lactate dehydrogenase, glucose-6-phosphatase and fructose 1, 6- bisphosphatase activities in kidney of alloxan induced diabetic rats. After 45 days the administration of aqueous extracts of flower and root of *A. lanata* (200 mg/kg bw) to diabetic rats showed decreased levels of glycolytic enzymes such as hexokinase, pyruvate kinase and lactate dehydrogenase and decreased levels of enzymes of HMP shunt and key enzymes of gluconeogenic pathway in kidney. Among the both extracts, the root of *A. lanata* was showed higher activity than flower extract.

The effect of aqueous extracts of flower and root of *A. lanata* on the levels of liver glycogen and glycogen metabolic enzymes like glycogen synthase and glycogen phosphorylase were studied and the results were represented in (Table 4). Alloxan induced diabetic rats showed significantly reduced levels of glycogen content in liver tissue. After 45 days, the oral administration of flower and root extract of *A. lanata* significantly increased the level of glycogen content in liver, which was as like as standard drug glibenclamide treated rats. Alloxan induced diabetic rats showed significantly decreased levesl of glycogen synthesis and the key enzyme like glycogen synthase and simultaneously increased glycogen degradation and the main enzyme glycogen phosphorylase in liver, which is due to decreased insulin activity and increased glucagon level in liver cells. The administration of flower and root extracts of *A. lanata* to diabetic rats showed reverse action of these key enzymes of glycogen metabolism.

Discussion

Over production of blood glucose by means of excessive hepatic glycogenolysis and gluconeogenesis and decreased utilization of glucose by the tissues is one of the fundamental basic of hyperglycemia in diabetes mellitus [33]. The flower and root of *A. lanata* treated diabetic rats showed that decreased levels of blood glucose and it may be due to increased production of insulin in beta cells of pancreas and increased utilization of glucose by tissues. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are excellent markers for diagnostic purpose, which play a main role in the conversion of amino acid to keto acid. AST was found in many tissues like liver, kidney, heart, brain and skeletal muscle and it is not specific liver enzyme, but ALT is more specific liver enzyme found in large amount in liver when compared to other tissues. Moreover, ALT and AST are marker enzymes for liver function and integrity [34], which are liberated into the blood whenever liver cells are damaged and the serum enzyme activity is increased. Similarly, in this study the increased levels of ALT and AST were observed in diabetic control. Hence it indicates that diabetes may induce hepatic dysfunction in rats and these elevations are also associated with cell necrosis of many tissues. The increase in the activities of ALT and AST in serum may be due to the leakage of the enzymes from liver cytosol to blood stream which gives an indication of the hepatotoxic of alloxan. The restoration of normal levels of these enzymes indicates normal functioning of liver under effective treatment [35]. The elevation of liver biomarker enzymes such as AST and ALT in alloxan induced diabetic rats that lead to leakage of enzymes, which indicates the liver damage [36]. Increased activities

Table 3. Effect of flower and root extracts of *A. lanata* on metabolic enzymes like hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and fructose-1,6-bisphosphatase in kidney. (Values are expressed as means ± S.E. of six samples from each group,Values are not sharing a common superscript differ significantly at 5% level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT)).

Groups	Hexokinase (µmoles of glucose -6-phosphate formed / hr/mg of protein)	Pyruvate kinase (µmoles of pyruvate formed/min/mg of protein	Lactate dehydrogenase (µmoles of pyruvate formed/hr/mg of protein)	Glucose-6-phosphate dehydrogenase (U/min/mg of protein)	Glucose-6-phosphatase (µmoles of phosphate liberated/hr/mg of protein)	Fructose-1,6-bisphosphates (µmoles of phosphate liberated/hr/mg of protein)
Group I Normal control	143.00±5.61^{a}	9.2±0.36^{a}	483.99±19.01^{a}	1.10±0.04^{a}	300±10.01^{a}	510±20.07^{a}
Group II Diabetic control	290.19±11.4^{c}	24.53±0.96^{d}	715.83±28.13^{b}	1.65±0.06^{c}	590±20.06^{d}	1980±70.58^{c}
Group III Diabetic + Flower extract (200mg/kg bw)	173.6±6.82^{b}	15.3±0.6^{c}	518.77±20.38^{a}	1.32±0.05^{b}	420±10.05^{c}	990±30.04^{b}
Group IV Diabetic + Root extract (200mg/kg bw)	157.59±6.19^{a,b}	10.95±0.43^{a,b}	509.18±20.00^{a}	1.13±0.04^{a}	350±10.18^{b}	610±20.04^{a}
Group V Diabetic + Glibenclamide (1mg/kg bw)	169.11±6.64^{b}	11.77±0.42^{b}	525.6±20.65^{a}	1.08±0.04^{a}	290±10.01^{a}	490±10.04^{a}

Table 4. Effect of flower and root extracts of *A. lanata* on glycogen, glycogen synthase and glycogen phosphorylase in liver. (Values are expressed as means ± S.E. of six samples from each group, Values are not sharing a common superscript differ significantly at 5% level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT)).

Groups	Liver glycogen (mg of glucose/g of tissue)	Glycogen synthase (µmoles of UDP formed /hr/ mg of protein)	Glycogen phosphorylase (µmole of phosphate liberated/hr/mg of protein)
Group I Normal control	41.61±1.63^{c,d}	865.17±33.99^{c}	667.19±26.21^{b}
Group II Diabetic control	22.84±0.89^{a}	575.49±22.61^{a}	899.80±3.53^{a}
Group III Diabetic + Flower extract (200mg/kg bw)	27.72±1.08^{b}	701.87±27.58^{b}	807.95±31.75^{c}
Group IV Diabetic + Root extract (200mg/kg bw)	40.65±1.59^{c}	836.4±32.86^{c}	735.63±28.9^{b,c}
Group V Diabetic + Glibenclamide (1mg/kg bw)	45.69±1.79^{d}	806.04±31.67^{c}	749.12±29.43^{c}

of transaminases which are active in the absence of insulin because of increased availability of amino acids in diabetes are due to increased break down of protein. The elevated transaminases activities were significantly reduced in aqueous extracts of flower and root of *A. lanata* treated diabetic rats. The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated levels of transaminase activity [37].

γGT catalyzes the transfer of γ-gutamyl group from γ-glutamyl peptides to another peptide or L-amino acids or water. The assay of γGT is a helpful adjunct in detecting hepatic damage. In this study, a significant elevation of the activity of γGT was observed in serum of alloxan induced diabetic rats and this is in accordance with earlier investigation [38]. The elevated activity of γGT in serum takes place as a result of hepatic induction of enzyme. In addition, hepatocelluar damage or cholestasis may also contribute to the elevated enzyme activity. A significantly increased level of γGT was also observed in untreated diabetic rats. γGT is found in hepatocytes and biliary epithelial cells and, can leak to blood in pancreatic disease, renal failure, and diabetes [39]. After 45 days, the oral administration of flower and root extracts of *A.lanata* to alloxan induced diabetic rats showed significantly decreased level of γGT activity.

Liver is the candidate organ involved in glucose homeostasis. It is the main site for glycolysis, a process where glucose is degraded and gluconeogenesis, where glucose is synthesized from lactate, amino acids and glycerol. The glycolysis and gluconeogenesis are important metabolic pathways that balance the glucose load in our body [40]. It plays an important role in the maintenance of blood glucose level by regulating its metabolism. Hexokinase, which brings about the first phosphorylation step of glucose metabolism, is reduced significantly in diabetic rats [41]. Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including hexokinase and pyruvate kinase. Hexokinase is universally present in cells of all types. Hexokinase catalyses the conversion of glucose to glucose -6-phosphate and plays a control role in the maintenances of glucose homeostasis. In the liver, the hexokinase is an important regulator of glucose storage and disposal [42]. The activity of hexokinase was significantly reduced in the liver of diabetic rats resulting in the diminished utilization of glucose and it leads to increased amount of glucose [43]. The increased level of hexokinase activity was observed in aqueous extracts of flower and root of *A. lanata* treated diabetic rats and is suggested that the activation of glycolysis leading to great utilization of glucose in liver cell.

During diabetes, liver shows decrease in weight due to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis, which is the outcome of lack of insulin and /or cellular glucose in liver cells [44]. This may be the reason for the diminished consumption of glucose in the system and increased blood glucose level. In alloxan induced diabetic rats, the insulin administration stimulated transcription of hexokinase mRNA synthesis and this enhanced the rate of synthesis and activity of the enzyme [45]. So, in this study the mechanism may be played by *A. lanata* extract in enhancing the hexokinase activity in diabetic treated rats.

Pyruvate kinase is a key enzyme of the glycolytic pathway whose activity and mRNA levels in the liver fluctuate according to the dietary status. Enzyme activity and mRNA concentration n strongly decrease with fasting and strongly increase after refeeding a high carbohydrate diet [46]. In vivo studies have shown that the expression of the pyruvate kinase gene is regulated by hormones and carbohydrates at both transcriptional and post-transcriptional levels. The major negative effector is glucagon and its second messenger, cyclic AMP; the major positive effectors are carbohydrates in the presence of insulin, thyroid hormones, and glucocorticoids which appear to play a "permissive" role [47,48]. Noguchi et al. [49] reported the pyruvate kinase is regulated at mRNA levels in insulin dependent diabetes. The role of pyruvate kinase is catalyzes the conversion of phosphoenolpyruvate to pyruvate. The present study is also showed that the increased level of pyruvate kinase in diabetic rats treated with flower and root of *A. lanata*. Similar result was observed in mangiferin and glibenclamide treated diabetic rats where increased the activity of pyruvate kinase that may leads to increase the utilization of glucose [50]. The findings of this study suggested that the flower and root of *A. lanata* showed the improvement in the glucose metabolism by increase the utilization of glucose.The activity of glucose-6-phosphate dehydrogenase, the rate limiting enzyme of the pentose phosphate pathway oxidative reaction was depressed in the liver of postmitochondiral fraction in diabetic rats. Zhang et al. [51] observed that increased level of glucose caused inhibition of glucose-6-phosphate dehydrogenase.

Lactate dehydrogenase in anaerobic glycolysis, catalyses the conversion of pyruvate to lactate which subsequently is converted to glucose in gluconeogenesis flux and the reaction occurs in both cytosolic and mitochondrial compartments. LDH activity is found to be altered by insulin, glucose, NADH, as well as increases in mitochondrial membrane potential, cytosolic free ATP and cytosolic free Ca2+ [52]. The decreased level of activity of LDH in tissues could be important to ensure that a high proportion of both pyruvate and NADH, supplied by glycolysis, is subsequently oxidized by mitochondria. This excessive pyruvate is converted to lactate for which LDH is needed and therefore the activity of LDH may be increased due to less insulin availability in diabetes [53,54].

In diabetic condition, an increased activity of lactate dehydrogenase was observed [55,56]. Lactate dehydrogenase and aldolase are the bifunctional enzymes involved in the glycolytic pathway [57]. The LDH system reflects the nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide (NAD+/ NADH) ratio indicated by lactate/pyruvate ratio of hepatocyte cytosol [58]. The activity of LDH was increased in diabetes due to glucose stimulated insulin secretion [59]. The *A. lanata* and glibenclamide treated diabetic rats were showed normal LDH activity and this may be regulation of NAD+/NADH ratio by glucose oxidation.

The kidney is involved in the regulation of glucose homeostasis and abnormalities were found in diabetes mellitus via three different mechanisms such as (i) release of glucose into the circulation via gluconeogenesis (ii) uptake of glucose from the circulation to satisfy its energy needs and (iii) reabsorption into the circulation of glucose from glomerular filtrate to conserve glucose carbon [60]. During diabetes, kidney shows increase in weight due to glucose–over utilization and subsequent enhancement in glycogen synthesis, lipogenesis and protein synthesis [44]. These changes may be leads to series of microvascular renal complications, which involves a series of metabolic changes in the pathogenesis of diabetic nephropathy. In kidney, an over utilization of cellular glucose occurs through elevated activities of glycolytic and NADP-linked lipogenic enzymes such as glucose-6-phosphate dehydrogenase, malic enzymes and isocitrate dehydrogenase (ICDH) [61,62].

Kidney is insulin independent tissue for the uptake of glucose and this primarily reflects the differences in their ability to metabolize cellular glucose. As described earlier, in the diabetic condition the

theory of glucose over and under utilization by the peripheral tissues plays a central role in disorder of glucose metabolism leading to elevated systemic glucose. In kidney, overutilization of cellular glucose occurs through elevated activities of the glycolytic enzymes. However renal gluconeogenic enzymes are elevated, similar to hepatic gluconeogenic enzymes thereby correlated to the uncontrolled glucose homeostasis at both cellular and systemic level [63].

The kidney can be considered two separate organs because glucose utilization occurs predominantly in the renal medulla, whereas glucose release is confined to the renal cortex [64]. This functional partition is a result of differences in the distribution of various enzymes along the nephron. For example, cells in the renal medulla have appreciable glucose phosphorylating and glycolytic enzyme activity and like the brain, they are obligate user of glucose [65]. These cells however, lack glucose-6-phosphate dehydrogenase and other gluconeogenic enzymes. Thus, although they can take up, phosphorylation of glycolysis and accumulate glycogen, they cannot release free glucose into the circulation [64]. The cytotoxicity of xenobiotics can be evaluated using serum marker enzymes. One such enzyme is lactate dehydrogenase, which though distributed throughout the body, it possesses isoenzymes recognized as marker for liver muscle lesion. It is important to exclude the possibility that the diabetes itself damage the tissue in such a way that activities of lactate dehydrogenase are attached [66]. Elevated serum lactate dehydrogenase levels in diabetic rats indicate cardiac muscular damage [67]. The quantity of enzyme released from damaged tissue is a measure of the number of necrotic cells.

The activity of glucose-6-phosphate dehydrogenase was increased in kidney of diabetic rats, which might lead to renal hypertrophy [68]. The significant changes in glucose-6-phosphate dehydrogenase lead to effect on cell growth and cell death [69]. The activity of glucose-6-phosphate dehydrogenase was increased in diabetes that might result in modulating the functioning of HMP shunt and the production of reducing equivalents such as NADH and NADPH. In this study the flower and root extracts of *A. lanata* treated diabetic rats, glucose-6-phosphate dehydrogenase activity was significantly reduced to near normal.

For the assessment of antidiabetic potency of the plant extract measured the activity of hepatic and kidney fructose 1, 6 bisphosphatase and glucose -6-phosphatase are important regulatory enzymes in gluconeogenesis. The final step of glucose production is catalyzed by glucose-6-phosphatase in kidney and liver. So, the glucose-6-phosphatase has an important role in glucose homeostasis in kidney and liver [70]. During the diabetic condition, fructose 1, 6- bis phosphatase hydrolyzes the fructose 1, 6- bisphosphate into fructose -6-phosphate in the gluconeogenesis [71]. In diabetic animals, the glucose-6-phosphatase and fructose 1, 6-bisphosphatase levels were observed to increase in kidney [72]. The increased activities of glucose-6-phosphatase and fructose 1, 6 bisphosphatase in liver and kidney of the alloxan induced diabetic rats may be due to insulin deficiency. In type I and type II diabetes the excessive release of glucose into the circulation is a major factor responsible for fasting hyperglycemia. Increased renal gluconeogenic enzymes activity [73] and increased renal glucose release have been consistently demonstrated in studies of diabetic animals [74].

Administration of alloxan causes decrease in glycogen content due to enhanced glycogenolysis which is due to insulin deficiency. So, the normal capacity of the liver to synthesize glycogen is impaired [75]. Restoration in glycogen level with treatment may be explained by the recovery of insulin and insulin control the activity of glycogen synthetase [76]. Glycogen synthesis in the rat liver and skeletal muscle is impaired in diabetes [77]. Glycogen is primary intracellular storable form of glucose and its level in various tissues especially skeletal muscles are a direct reflection of insulin activity because insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since alloxan causes selective destruction of β cells of islet of langerhans resulting in marked decrease in insulin level, it is rational that glycogen level in liver and muscles are decreased as they depend on insulin for influx of glucose [78].

Conclusion

The present study showed that the flower and root extracts of *A. lanata* regulate the carbohydrate metabolic enzymes activities in alloxan induced diabetic rats. The extracts of flower and root of *A. lanata* was significantly maintained the levels of carbohydrate metabolic enzymes such as aerobic and anaerobic glycolytic key enzymes, HMP shunt, gluconeogenic key enzymes and glycogen metabolic enzymes were restored to near normal levels in liver and kidney. The higher level of antidiabetic activity was observed in root extract of *A. lanata* treated diabetic rats than flower extract. These findings suggest that the *A. lanata* may be a potential source for the development of new oral hypoglycemic drugs.

Conflicts of Interest

The authors declare no conflict of interest in this study.

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