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Amelioration of carbohydrate and fat metabolism by *Houttuynia cordata* and *Hypochaeris radicata* in alloxan-induced diabetic mice

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Abstract

Purpose: *Houttuynia cordata* and *Hypochaeris radicata,* local wild edibles were evaluated for their antidiabetic activity and effect on glycolytic enzymes. The activities of these plant extracts were compared to the anti-diabetic drug glibenclamide.
Methods: Mice were divided into control and test groups and were treated on every alternate day for a period of 14 days. Following the 14th day, serum and tissues were collected. For sub-acute studies and lipid profiling, serum was used while liver and skeletal muscle tissues were used for glycogen content estimation and glycolytic enzyme assays. The expression of glycolytic enzymes in normal, diabetic and diabetic treated mice was quantified using Kodak Digital Science 1 D Image Analysis Software, Version 3.0.
Results: The oral administration of extracts of *Houttuynia cordata* and *Hypochaeris radicata* lead to a significant blood glucose reduction. *Houttuynia cordata* and *Hypochaeris radicata* significantly lowered serum alanine transaminase (ALT) and aspartate transaminase (AST), triglyceride, total cholesterol, and low-density lipoprotein-cholesterol (LDL) levels of diabetic mice. Elevated high-density lipoprotein-cholesterol (HDL) was significantly increased upon administration of the extracts. Altered glycogen content seen in diabetics was also restored to near-normal levels. The plant extracts also enhanced the activity of the glycolytic enzymes.
Conclusion: From the present study, it can be concluded that *Houttuynia cordata* and *Hypochaeris radicata* have potent anti-diabetic properties.
Keywords: Diabetes, glycolytic enzymes, Houttuynia cordata, Hypochaeris radicata, lipids..

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1. Introduction

Diabetes mellitus, commonly referred to as diabetes, is a group of chronic metabolic conditions and one of the major non-communicable diseases today. According to the International Diabetes Federation, in 2019 463 million people were diabetic and this is projected to increase to 700 million by 2045 (International Diabetes Federation, 2019). India has the second-largest diabetes population (74 million) after China [1]. In the northeastern states of India, Meghalaya has the highest population of diabetes (9.89%) followed by Mizoram and Tripura (9.4%), Manipur (8.8%), and Sikkim with (7.8%) in case of women whereas in case of men Nagaland (11.1%) is the second leading state with diabetics after Meghalaya (12.5%) followed by Mizoram and Sikkim with (10.7%) [2,3].

Diabetes is characterized physiologically by the deficiency in insulin or insulin activity and clinically by hyperglycemia or impaired glucose tolerance [4]. As it progresses, tissue or vascular damage ensues leading to severe diabetic complications [4]. Diabetes-related complications including cardiovascular disease, kidney disease, neuropathy, and blindness are significant causes of increased morbidity and mortality and result in a substantial

economic burden [5]. Therefore, its management is considered a global problem, and successful treatment to achieve glycemic control remains a cherished goal. Available drugs for the treatment of diabetes include insulin analogs and secretagogues, GLP-1 analogs, DPPIV inhibitors, SGLT2 inhibitors, and biguanides [6]. However, though valuable in achieving glycemic control, the drugs have adverse side effects associated with them whether administered singly or in combination [7]. Hence, there is a need for safer agents. Plants and herbal drug preparations have been used traditionally for the treatment of various ailments since time immemorial. Medicinal plants have the advantage of having no or minimal side effects [8].

Several plants are reported to employ different mechanisms for reducing blood glucose levels. Flavonoids, terpenes, alkaloids, saponins, tannins, and other secondary phytochemicals are responsible for the therapeutic value of these plants [9]. There are those which exhibit properties similar to well-known sulfonylureas like glibenclamide affecting hypoglycemia by stimulating insulin release from pancreatic β-cells [10], while others act like biguanides, specifically metformin [11] which inhibits gluconeogenesis. *Zaleya decanda* [12] and *Acacia Arabica* [12] exhibit hypoglycemic activity by enhancing insulin secretion and insulin activity and inhibiting oxidative stress. In the biodiversity-rich areas of the Northeastern part of India, the local communities have traditionally used and relied on herbs for treating various ailments. However, given the vast repertoire of medicinal plants used by the indigenous communities today only a few of the traditional plants have received scientific scrutiny vis-à-vis anti-diabetic properties such as *Potentilla fulgens I* L. [13], *Osbeckia chinesis* [14], *Flemingia macrophylla L.* [15], *Smilax perfiolata* [16] and *Ixeris gracilis* [17]. Therefore, our study with wild edible plant extracts continues and will be useful if their efficacy, mechanism of action, and safety are determined.

In North-East India, *Houttuynia cordata* and *Hypochaeris radicata* are medicinal herbs that are eaten as a salad. Anti-inflammatory, anticancer, anaphylactic inhibitory, antioxidant, antibacterial, antifungal, anti-allergic, anti-obesity, and antidiuretic qualities are claimed to be present throughout the plant [18,19].

In the traditional medical practice of Tamil Nadu, *H. radicata* is used to treat jaundice, rheumatism, dyspepsia, constipation, hypoglycemia, and kidney-related issues [20]. Furthermore, the leaf juice of *H. cordata* is used to cure cholera, dysentery, blood insufficiency, and blood purification [21]. However, there hasn't been much scientific support for the medical properties of these plants. Hence, the current investigation was carried out to fill this need.

2. Materials and methods

2.1 Chemicals

Alloxan, Bovine serum albumin (BSA), Nicotinamide adenine dinucleotide phosphate (NADP), Adenosine triphosphate (ATP), Glucose-6-phosphate dehydrogenase, Nitrocellulose membrane (0.045 μm pore size), N,N,N',N'- tetramethylene diamine (TEMED), β-mercaptoethanol were procured from Sigma Co. USA. Primary antibodies for glucokinase and hexokinase were obtained from Santacruz Biotechnology, USA. Tween 20, Horseradish conjugated rabbit anti-mouse IgG, 3,3', 5,5'-tetramethylbenzidine/hydrogen peroxide (TMB/H2O2) were from Bangalore Genei. Standard drug metformin (glycomet) was obtained from USV Pharmaceuticals Limited, India, Glibenclamide (daonil) from Aventis Pharma Limited, India. SD check glucometer and glucostix were from BD Biosensor, Korea. All other chemicals used were of analytical grade purchased from Merck, Himedia, and Sisco Research Limited, India.

2.2 Plant material

Leaves of *Houttuynia cordata* (Voucher no. NEHU-11922) were collected from Mairang village, Meghalaya. Leaves of *Hypochaeris radicata* (Voucher no. NEHU-11967) were collected from North-Eastern Hill University, Shillong Campus, Meghalaya. Plant specimens were submitted and identified by herbarium curator Dr. P.B. Gurung, Department of Botany, North-Eastern Hill University, Shillong, Meghalaya.

2.3 Plant extraction

Fresh leaves were washed, dried, and powdered using an electric blender. 1 Kg of dried powder was 10L of distilled water for 24h [22]. Following this, the mixture was filtered using a muslin cloth followed by whatman no.1. The filtrate was lyophilized (ScanvacCoolsafe) to obtain the crude powder (gm). The crude powder was weighed to calculate the percentage yield and stored at 4^{O}C for further usage.

2.4 Experimental animals

Healthy, adult Swiss Albino mice weighing 25-30 g purchased from Pasteur Institute, Shillong was used for the study. The mice were housed in a room maintained under controlled conditions with temperature maintained at 25^{O}C on a 12h light/dark cycle and fed with balanced mice feed obtained from Amrut Laboratory, Pune, India. This study was performed in compliance with the Institutional guidelines dated 4th Dec 2014.

2.5 Preparation of diabetic mice

Mice were administered with alloxan monohydrate dissolved in acetate buffer (0.15M, pH 4.5) intraperitoneally at a dose of 180 mg/kg b.w [12]. Before induction, mice were fasted overnight but given *ad libitum.* The animals were kept under observation for a week after induction and blood

glucose was subsequently determined. Mice with fasting blood glucose above 200 mg/dL were considered diabetic and used for the experiments. Following a period of treatment for 14 days, mice were sacrificed and tissues were excised for the experiments.

2.6 Glucose tolerance test (GTT)

Normoglycemic and alloxan-induced diabetic mice fasted overnight but provided water *ad libitum* were tested for glucose tolerance. Mice were divided into five tests and one control group. Test groups were administered with one of the following: *H. cordata* extract (450mg/kg b.w), *H.radicata* extract (250mg/kg b.w), metformin (500mg/kg b.w), glibenclamide (10mg/kg b.w) and insulin (10U/kg b.w). 2h before intraperitoneal glucose load of 2g/kg b.w, the extracts and standard drugs were administered to the various groups of mice. Glucose level was measured before administration and subsequently at 30, 60, and 120 min after the glucose load. Mice were then fed normally and blood glucose was recorded at 1440 min (24h). Control groups were treated similarly.

2.7 Sub-acute studies

2.7.1 Alanine transaminase (ALT) and Aspartate transaminase (AST) assay

The activity of alanine transaminase (ALT: EC 2.6.1.2) and aspartate transaminase (AST: EC 2.6.1.1) in the serum was determined using commercially available kits from Coral Diagnostics, India. Assays were performed using a 3000 Evolution Blood Analyzer.

2.8 Lipid profile

2.8.1 Triglycerides estimation

Serum was reacted with 50mmol/L Pipes buffer, 5mmol/L 4-chlorophenol, 5 mmol/L magnesium ion, 1 mmol/L ATP, 5000 U/L lipase, 1000 U/L peroxidase, 400 U/L glycerol kinase, 0.4 mmol/L4-aminoantipyrene and 4000 U/L glycerol-3-phosphate oxidase. The solution was mixed well, incubated at 370C for 10 min and the absorbance was measured against a blank at λ505nm. The results were calculated as:

Triglycerides (mg/dL) = Absorbance of test/ Absorbance of standard X 200

2.8.2 Total cholesterol estimation

Serum was reacted with 50 mmol/L Good's buffer (pH 6.7), 50U/L cholesterol oxidase, 100U/L cholesterol esterase, 3 KU/L, peroxidase, and 0.4 mmol/L –amino antipyrine. The solution was mixed well, incubated at 37^0C for 10 min and the absorbance was measured against a blank at λ505nm. The results were calculated as above.

2.8.3 HDL-cholesterol and LDL-cholesterol estimation

The serum was first precipitated with polyethylene glycol 6000 and centrifuged. The resultant supernatant was then reacted with 50 mmol/L Good's buffer (pH 6.7), 50U/L cholesterol oxidase, 100U/L cholesterol esterase, and 3 KU/L peroxidases, and 0.4 mmol/L amino antipyrine. The solution was mixed well, incubated at 37^0C for 10 min and the absorbance was measured against a blank at λ505nm. The HDL-cholesterol concentration was calculated as above whereas the LDL-cholesterol concentration was calculated as:

LDL-cholesterol (mg/dL) =

Total cholesterol – triglycerides/5 – HDL-cholesterol

2.9 Glycogen content

Liver, skeletal muscle, and kidney tissues were homogenized in 5% TCA (3ml/gm) and centrifuged at 3000 x g for 10 min at 4°C. The supernatant was collected and 45% ethanol was added and refrigerated overnight. Glycogen was then obtained from the supernatant solution after centrifugation and then estimated using the anthrone method.

2.10. Glucokinase and hexokinase assay

Glucokinase and hexokinase activity was assayed using 100mM Tris-HCl (pH 7.5), 5mM $mgCl_2$, 5mM ATP, 100mM Glucose and 0.2mM NADP. This was then mixed with the reaction cocktail (Glucose-6-phosphate dehydrogenase and tissue extract) by inversion and equilibrated at 30^0C using a Peltier temperature controller followed by spectroscopic measurement at λ340nm. The change in absorbance per min (Δ) was monitored spectrophotometrically and the absorbance was measured at λ340nm. The volume activity and specific activity were calculated as:

Volume activity= 3/ 6.22x 1.0 x0.1 X ΔA/min [U/ml sample]

6.22= molar extinction of NADPH at λ340nm

1.0= path length in centimeters

0.1= volume of tissue extract taken in millimeters

Specific activity = volume activity/ concentration of protein X [U/mg protein]

2.11. Western blot analysis

Protein levels of glucokinase in the liver and hexokinase in the liver and muscle were detected by western blot using SDS-PAGE. Following electrophoresis, the proteins were transferred to a nitrocellulose membrane (NCM) and transferred at 100V for 1h at 4^oC. The blotted membrane was then blocked using a blocking solution (5% skimmed milk in TBS) for 1h. Following blocking, the blot was incubated overnight with primary antibodies for glucokinase, hexokinase, and β-actin. The membrane was washed in tween tris buffer saline (TTBS) to remove unbound antibodies and incubated with anti IgG conjugated to horseradish peroxidase for 3h followed by the addition of TMB/H_2O_2 substrate for color development. The reaction was stopped by washing the membrane in distilled water. The blot was quantified using Kodak Digital Science 1 D Image Analysis Software, Version 3.0.

2.12. Statistical analysis

The data were evaluated using a one-way analysis of variance (ANOVA) and the findings were presented as mean ± SEM.

3. Results

3.1. Glucose tolerance test (GTT)

Treatment with *H.cordata* (450 mg/kg b.w) and *H.radicata* (250 mg/kg b.w) aqueous extract 2h before glucose load improved glucose tolerance in overnight fasted normoglycemic and alloxan-induced diabetic mice. Significantly, in normoglycemic mice, the suppression of the glucose peak by *H.cordata* was comparable to that of glibenclamide whereas the suppression of glucose peak by *H.radicata* was not as high as glibenclamide (**Fig.**1a).

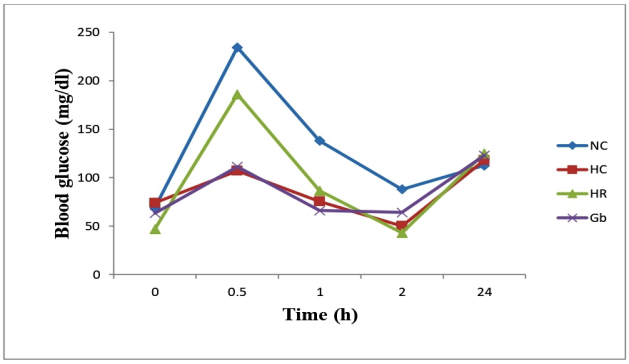

Fig. 1(a): Glucose tolerance test in normal mice administered with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide.
Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; M: Mean; SEM: Standard Error Mean; NC: Normal control; HC: normal mice treated with *H.cordata*; HR: normal mice treated with *H.radicata*; Gb: normal mice treated with Glibenclamide.

In alloxan-induced diabetic mice, *H. cordata* significantly suppressed the glucose peak (61%, p<0.001; 80%, p<0.001and 82%, p<0.001 at 0.5, 1 and 2 h respectively). The effect was greater than that of glibenclamide (**Fig.**1b).

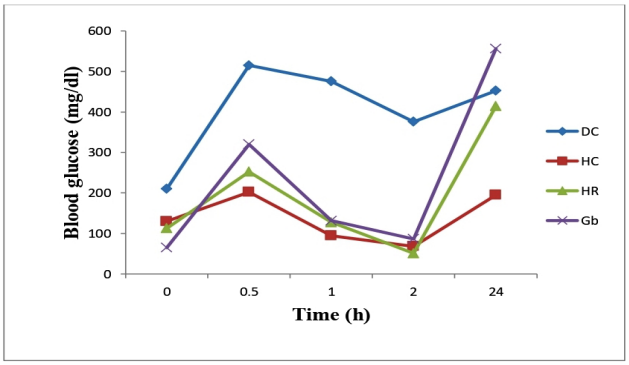

Fig. 1(b): Glucose tolerance test in alloxan-induced diabetic mice administered with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide.
Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; M: Mean; SEM: Standard Error Mean; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

H.radicata also significantly suppressed the glucose peak (50%, p<0.001; 73%, p<0.001and 86%, p<0.001 at 0.5, 1 and 2 h respectively). The effect was seen to resemble that of glibenclamide concerning the magnitude and pattern of glucose suppression.

3.2. Sub-acute studies

3.2.1. Alanine transaminase (ALT) and Aspartate transaminase (AST) assay

Alloxan-induced diabetic mice showed a significant increase in ALT (66%, p<0.001) and AST (65%,p<0.01) activity when compared to the normal control. Notably, *H.cordata* (20%, p<0.05), *H.radicata* (34%, p<0.01) and glibenclamide (32%, p<0.01) resulted in significant lowering of ALT activity from that of the diabetic group (**Fig**.2a). The elevated AST (65%, p<0.01) was effectively lowered by *H.radicata* (21%, p<0.05). *H.cordata* and glibenclamide on the other hand did not show any significant lowering of AST activity (**Fig**.2b).

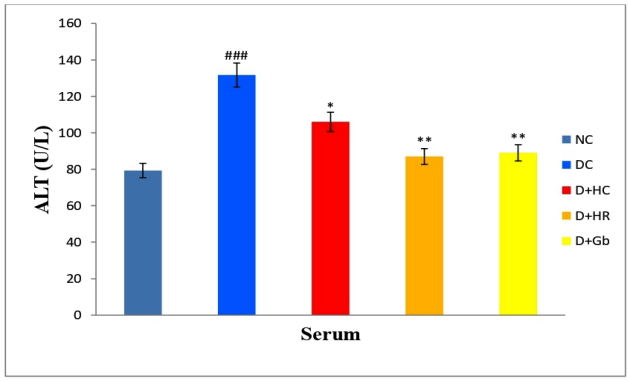

Fig. 2(a): Serum ALT activity of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide.
Values are expressed as M ± SEM. Meana are obtained from 6 separate experiments; p: #represents level of significance at ###p<0.001 compared against the normoglycemic group and * represents level of significance at **p<0.01, *p<0.05 compared against the diabetic group. M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

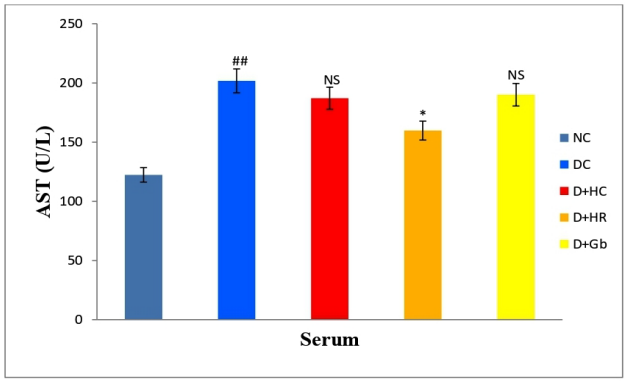

Fig. 2(b): Serum AST activity of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide.
Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; p: #represents level of significance at ##p<0.01 compared

against the normoglycemic group and * represents level of significance at*p<0.05 compared against the diabetic group. NS: non-significant: M: Mean; SEM: Standard Error Mean; Nc: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

3.3. Lipid profile:

3.3.1. Triglycerides estimation

Administration of *H.cordata* and glibenclamide resulted in a significant lowering of serum total glyceride level with a reduction of 42% (p<0.01) and 49% (p<0.001) respectively (**Fig.** 3a) *H.radicata* on the other hand did not show any significant lowering of triglycerides.

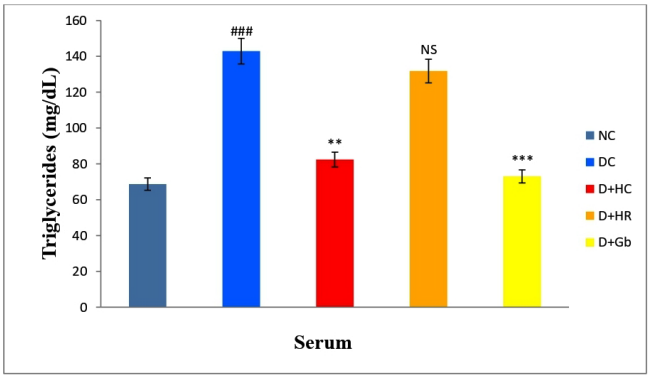

Fig. 3(a): Serum triglyceride level of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w.), *H.radicata* (450 mg/kg b.w.) and glibenclamide.

Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; p: #represents level of significance at ###p<0.001 compared against the normoglycemic group and * represents level of significance at ***p<0.001, **p<0.01 compared against the diabetic group. NS: Non-significant; M: Mean; SEM: Standard Error Mean; Nc: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

3.3.2. Total cholesterol estimation

Administration of *H.radicata, H.cordata* and glibenclamide resulted in significant lowering of serum total cholesterol level with a reduction of 10% (p<0.05), 13% (p<0.05) and 26% (p<0.01) respectively (**Fig.** 3b).

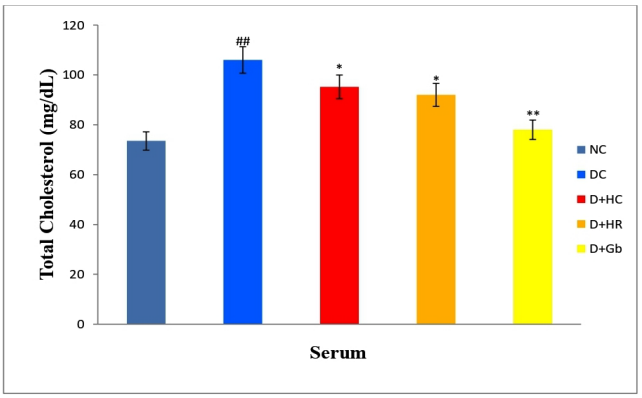

Fig. 3(b): Serum total cholesterol level of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w.), *H.radicata* (450 mg/kg b.w.) and glibenclamide.

Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; p: #represents level of significance at ##p<0.01 compared against the normoglycemic group and * represents level of significance at **p<0.01, *p<0.05 compared against the diabetic group. M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

3.3.3. HDL-cholesterol and LDL-cholesterol estimation

Administration of *H.radicata, H.cordata* and glibenclamide resulted in significant increase of serum HDL-cholesterol level with an increase of 79% (p<0.001), 95% (p<0.001) and 105% (p<0.001) respectively (**Fig.** 3c).

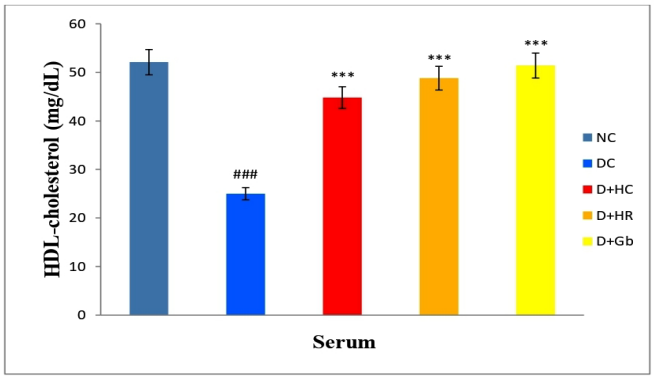

Fig. 3(c): Serum HDL cholesterol level of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w.), *H.radicata* (450 mg/kg b.w.) and glibenclamide.

Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; p: #represents level of significance at ###p<0.001 compared against the normoglycemic group and * represents level of significance at ***p<0.001 compared against the diabetic group. M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

The elevated LDL-cholesterol was effectively lowered by, *H.cordata* (36%, p<0.001), *H.radicata* (67%, p<0.001) and glibenclamide (76%, p<0.001) (**Fig.** 3d).

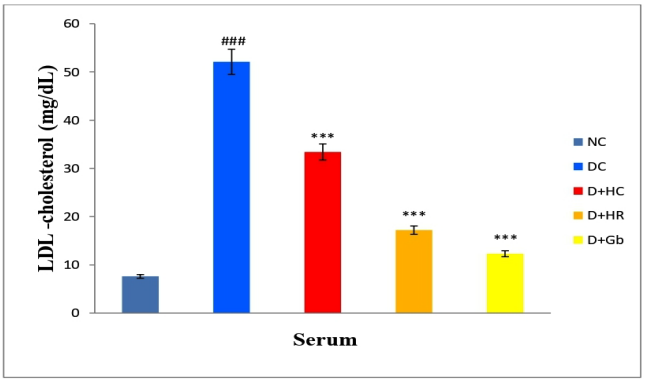

Fig. 3(d): Serum LDL cholesterol level of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide. Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; p: #represents level of significance at ###p<0.001 compared against the normoglycemic group and * represents level of significance at ***p<0.001 compared against the diabetic group. M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

3.4. Glycogen content

Alloxan-induced diabetic mice showed a significant decrease of hepatic (63%, p<0.001) and muscle (61%, p<0.001) glycogen when compared to the normal control. This reduction was remarkably elevated following treatment with *H.radicata, H.cordata* and glibenclamide. The increase was 115.9% (p<0.001), 151.8% (p<0.001) and 144.3% (p<0.001) in liver and 79.5% (p<0.05), 85.1% (p<0.01) and 126.1% (p<0.001) in skeletal muscle respectively (**Fig.** 4).

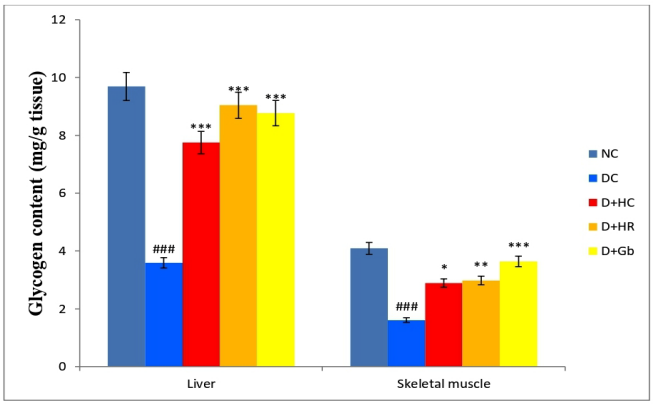

Fig. 4: Glycogen content in liver and skeletal muscle of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide.
Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; p: #represents level of significance at ###p<0.001 compared against the normoglycemic group and * represents level of significance at ***p<0.001, **p<0.01, *p<0.05 compared against the diabetic group. M: Mean; SEM: Standard Error Mean NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

3.5. Glucokinase (GK) and hexokinase (HK) activity

GK activity was substantially reduced in diabetic control with a reduction of 65% (p<0.001) from that of the normal control.

Treatment with *H.cordata* and *H.radicata* resulted in an increased GK activity. The increase was 159% (p<0.001) and 134% (p<0.01) respectively (**Fig.** 5a). Glibenclamide on the other hand did not show any significant effect on hepatic GK activity.

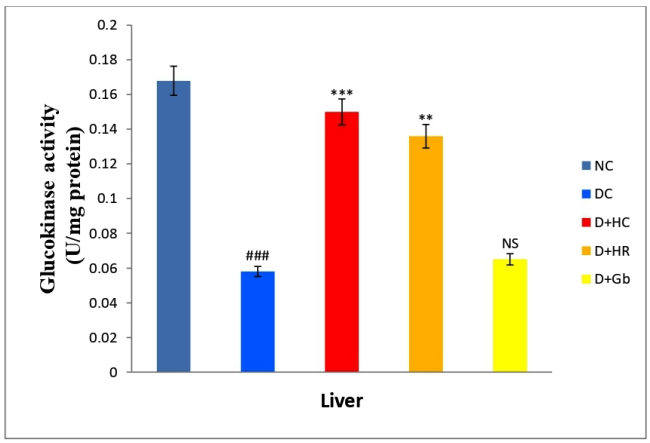

Fig. 5(a): Glucokinase activity (U/mg protein) in liver of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w.), *H.radicata* (450 mg/kg b.w.) and glibenclamide. Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; p: #represents level of significance at ###p<0.001 compared against the normoglycemic group and * represents level of significance at ***p<0.001, **p<0.01 compared against the diabetic group. NS: Non-significant; M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

HK activity in alloxan-induced mice significantly decreased in the liver (60%, p<0.01) and skeletal muscle (80%, p<0.001). Treatment with *H.cordata* and *H.radicata* resulted in an increased HK activity. The increase was 97% (p<0.05) and 157% (p<0.01) in liver while in skeletal muscle the increase was 258% (p<0.01) and 517% (p<0.01) respectively (**Fig.** 5b). Glibenclamide resulted in a significant enhancement only in the skeletal muscle with an increased enzyme activity of 17% (p<0.05).

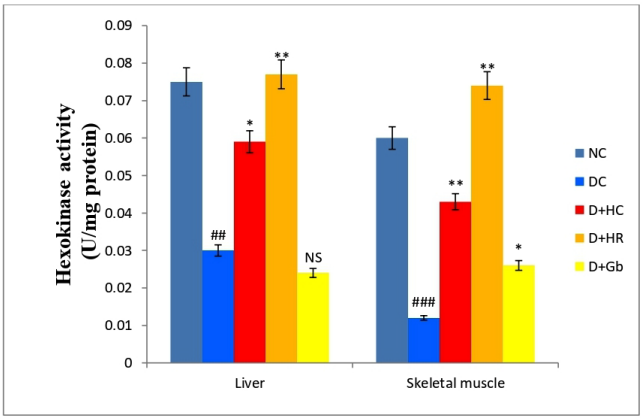

Fig. 5(b): Hexokinase activity (U/mg protein) in liver and skeletal muscle of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w.), *H.radicata* (450 mg/kg b.w.) and glibenclamide. Values are expressed as M ± SEM. Means are obtained from 6 separate experiments; p: #represents level of significance at ###p<0.001 compared against the normoglycemic group and * represents level of significance at **p<0.01, *p<0.05 compared against the diabetic group. NS: Non-significant; M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

3.6. Western blot analysis

In comparison to the normal group, the diabetic group showed a significant decrease (p<0.001) in the protein level of GK in the liver. Treatment with *H.cordata* and *H.radicata* reversed the effects, restoring the protein level to near-normal levels (p<0.001,p<0.01, respectively). No significant alteration in protein level was observed in the glibenclamide-treated diabetic group (**Fig.** 6).

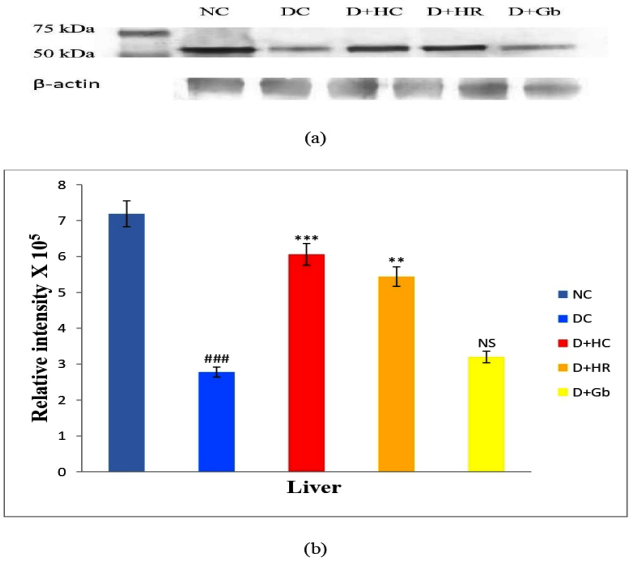

Fig. 6: Protein level of glucokinase activity in liver of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide. (a) Western blot analysis. (b) The relative intensity of the western blot determined by densitometric analysis (KDS-I software). Values are expressed as M ± SEM. Means are obtained from 3 separate experiments; p: #represents level of significance at ###p<0.001 compared against the normoglycemic group and * represents level of significance at ***p<0.001, **p<0.01 compared against the diabetic group. NS: Non-significant; M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

HK protein levels significantly decreased in the liver (p<0.05) and skeletal muscle (p<0.01) of alloxan-induced diabetic mice. The levels were significantly increased on treatment with *H.cordata* (p<0.05) and *H.radicata* (p<0.01) in the liver (**Fig.**7).

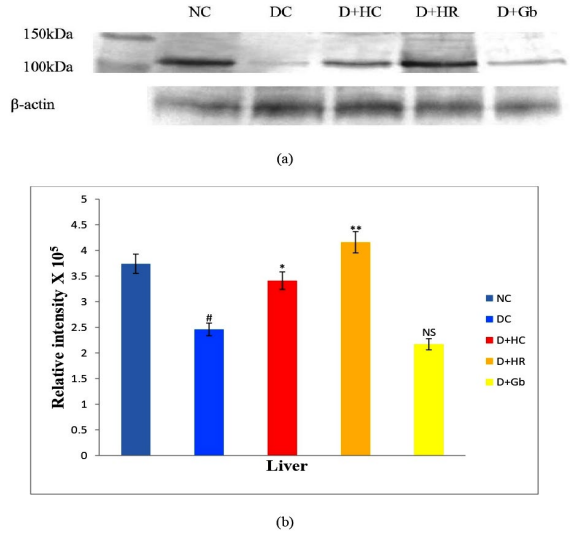

Fig. 7: Protein level of hexokinase activity in liver of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide. (a) Western blot analysis. (b) The relative intensity of the western blot determined by densitometric analysis (KDS-I software). Values are expressed as M ± SEM. Means are obtained from 3 separate experiments; p: #represents level of significance at #p<0.05 compared against the normoglycemic group and * represents level of significance at **p<0.01, *p<0.05 compared against the diabetic group. NS: Non significant; M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

No significant alteration in protein level was observed in the glibenclamide-treated diabetic group. In skeletal muscle, treatment with *H.cordata* (p<0.01), *H.radicata* (p<0.01), and glibenclamide (p<0.01) resulted in increased protein levels of the enzyme (**Fig.**8).

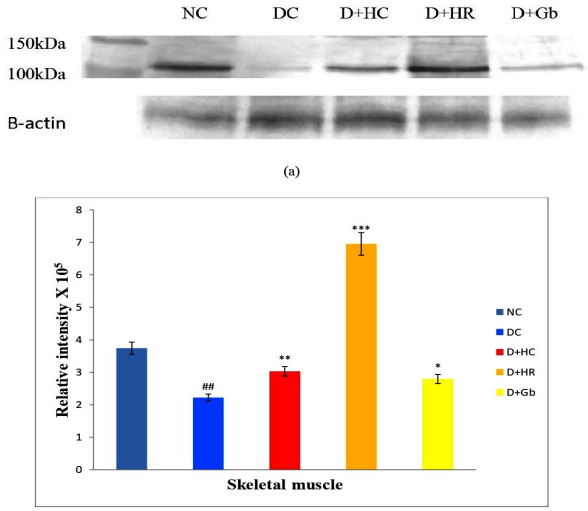

Fig. 8: Protein level of hexokinase activity in skeletal muscle of normoglycemic, alloxan-induced diabetic and alloxan-induced diabetic mice treated with *H.cordata* (450 mg/kg b.w), *H.radicata* (450 mg/kg b.w) and glibenclamide. (a) Western blot analysis. (b) The relative intensity of the western blot determined by densitometric analysis (KDS-I software). Values are expressed as M ± SEM. Means are obtained from 3 separate experiments; p: #represents level of significance at #p<0.05 compared against the normoglycemic group and * represents level of significance at ***p<0.001; **p<0.01, *p<0.05 compared against the diabetic group. NS: Non-significant; M: Mean; SEM: Standard Error Mean; NC: Normal control; DC: Diabetic control; D+HC: Diabetic mice treated with *H.cordata*; D+HR: Diabetic mice treated with *H.radicata*; D+Gb: Diabetic mice treated with Glibenclamide.

4. Discussion

Oral glucose tolerance is a gold standard in the diagnosis of diabetes mellitus. It reflects the extent of intestinal glucose absorption and hepatic glucose metabolism [22]. Studies have shown that impaired glucose tolerance is not only a risk factor for the progression of diabetes but also cardiovascular diseases [5]. Based on the results of the present study, aqueous extracts of *H.cordata* and *H.radicata*

were potent in suppressing the glucose peak. It was also observed that the glucose-lowering effect of the plant extracts is comparable to glibenclamide. This could be due to the presence of certain compounds that elicit glibenclamide-like action. Glibenclamide is a well-known insulin secretagogue causing hypoglycemia by stimulating pancreatic β-cells to release more insulin.

Elevated activity of ALT and AST is an indicator of liver disease and occurs in diabetics more frequently than in the general population [22]. The results obtained in the present study also support this report wherein the diabetic control mice were seen to display elevations in both ALT and AST activity (**Fig.** 2). It was observed that both the plant extracts tested were effective at varying degrees in lowering the elevated ALT levels. Elevated AST activity on the other hand was significantly lowered only by *H.radicata* treatment while *H.cordata* and glibenclamide had no significant effect. The effectiveness of *H.radicata* in restoring near-normal levels of ALT and AST activity needs further investigation. The adverse effect of abnormal lipid levels is associated with insulin resistance, glucose intolerance, obesity, hypertension, and accelerated atherosclerosis [23]. The high concentration of serum triglycerides in diabetic conditions is mainly due to an increase in mobilization of free fatty acids from the peripheral deposits since insulin inhibits lipase [24]. Under normal conditions, insulin activates lipoprotein lipase and hydrolyses triglycerides, whereas insulin deficiency fails to activate the enzyme thereby causing hypertriglyceridemia [25]. The results of the present study indicate that the lipid abnormality developed in diabetic conditions was significantly countered by treatment with the *H.cordata* as well as glibenclamide. *H.radicata* had no significant effect although it could lower the elevated lipid level. Studies indicated that flavonoids from the plants *Emblica officinalis* and *Mangifera indica* were effective against dyslipidemia [26]. Therefore, the lipid-lowering effect of *H.cordata* and *H.radicata* could to due to their flavonoid content.

One of the characteristic changes occurring in uncontrolled diabetes is the increase in glycogen breakdown of tissues that are normally dependent on insulin [27]. Glycogen is the primary intracellular storable form of glucose and its level in various tissues is a direct reflection of insulin activity [23]. Alloxan causes selective destruction of β-cell of the islet of Langerhans resulting in a marked decrease in insulin levels [28]. Glycogen level in the tissues decreases as they depend on insulin for the influx of glucose [23]. From the results obtained, the decrease in glycogen content in liver and skeletal muscle is consistent with earlier findings. Treatment with *H.cordata*, *H.radicata* as well as glibenclamide increased the glycogen content of both liver and skeletal muscle to near-normal levels. Notably, the effect of *H.radicata* was more pronounced than *H.cordata* in hepatic glycogen content. The significant accumulation of glycogen in the liver and skeletal muscle in the present study could have been possible due to either stimulation of insulin release from β-cells or due to an insulin-mimetic activity of the component of the extracts resulting in direct peripheral glucose uptake or due to a combination of the two [23].

The activity of GK and HK has been shown to be very sensitive indicators of the glycolytic pathway and this is decreased in the diabetic state. In conformity with other studies, our findings indicate that liver and skeletal muscle GK and HK activities were reduced in alloxan-induced diabetic mice (Figure 5a &5b). Alloxan is reported to inhibit GK and HK activity by binding to the SH group of the enzyme, inactivating it [29]. Further, insulin is known to regulate enzymes involved in carbohydrate metabolism and a major action of insulin in the liver is to stimulate hepatic GK expression [29]. The results of the present study indicate that the declined hepatic activities seen in diabetic mice were significantly elevated to near normal levels following treatment with both the extracts (Figure 5a, 5b, 6 & 7). While *H.cordata* seemed to be more effective in enhancing GK activity, the extract of *H.radicata* on the other hand was most promising in elevating hepatic HK activity. Glibenclamide had no significant effect in altering hepatic GK and HK activities. This result is supported by other studies where it was shown that the sulfonylurea did not significantly stimulate glucokinase gene expression in pancreatic β-cells. This may also be understood from the homeostatic and regulatory roles of the liver and that enzymes in tissues are differentially regulated. Further, alterations of skeletal muscle HK were significantly enhanced by *H.cordata*, *H.radicata,* and glibenclamide. An increase in the activity of GK and HK in the extract-treated mice implies that cellular entry of glucose was facilitated by the extracts, which in turn stimulated the activity of these enzymes.

5. Conclusion

Accordingly, based on the results of the present study it can be concluded that the wild edibles *H.cordata* and *H.radicata* have significant glucose-lowering properties and can be potential sources for antidiabetic drug mediating their effects by enhancing the activities of glucokinase and hexokinase, increasing HDL, lowering total triglyceride, total cholesterol, and LDL concentrations. Consumption of such wild edibles should be encouraged in the present day given the onslaught of the fast-food culture. Metabolic and proteomic studies on the active metabolite composition of the plants would enlighten the exact mechanism involved and help rationalize their use in addressing diabetes and its complications.

Conflict of interests

The authors declare no conflict of interests.

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