Biological Forum – An International Journal **15(5): 1655-1669(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Evaluation of Anti-diabetic and Hypolipidemic Activity of *Drypetes roxburghii* Roots

Isha Talwar[1], Satish Kumar Sharma[1*] and Kaneez Fatima[2]
[1] Glocal School of Pharmacy, Glocal University,
Delhi-Yamunotri Marg, SH 57, Mirzapur Pole Saharanpur (Uttar Pradesh), India.
[2] Faculty of Pharmacy, Maulana Azad University, Jodhpur (Rajasthan), India.

(Corresponding author: Satish Kumar Sharma)*
(Received: 05 March 2023; Revised: 25 April 2023; Accepted: 05 May 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Metabolic disorders such as diabetes (higher glucose level) and obesity (hyperlipidemia) is a major cause of cardiovascular disease. Apart from mainstream medications, botanicals serve as a popular treatment to manage these conditions. To evaluate anti-diabetic and hypolipidemic activity of *Drypetes roxburghii* (DR) root extract in experimental animal models.

The roots were extracted using 70% ethanol. Flavonoid and phenolic compound content were quantitatively estimated. Acute oral toxicity of the extract was determined and two doses were selected to evaluate the pharmacological activity. For evaluation of anti-diabetic activity streptozotocin induced diabetes was used as a model and parameters like blood glucose level, liver enzymes, in-vivo oxidative parameters, in-vitro α-amylase and α-glucosidase level were determined. For evaluation of hypolipidemic activity, high fat diet induced hyperlipidemia was used as a model and parameters like body weight, complete lipid profile and HMG-CoA reductase inhibition was determined. The phenolic content and the flavonoid content was found to be 55.740 µg GAE/mg and 142.87 µg QE/mg respectively, in crude extract. The extract was found to be safe till 2000 mg/kg BW. The extract at dose 200 mg/kg BW was found to be a potent anti-diabetic and anti-hyperlipidaemic agent. The extract showed potent anti-diabetic and hypolipidemic activity. Ethical concern, selection of statistical test.

Keywords: *Drypetes roxburghii*, Flavonoid content, Diabetes, hyperlipidemia, streptozotocin high-fat-diet.

INTRODUCTION

Hyperlipidemia and diabetes mellitus play essential roles in clinical medicine since they are both well-established cardiovascular risk factors with therapeutic benefit when treated. Many people with type 2 diabetes have dyslipidemia, which is thought to be crucial in moderating the cardiovascular risk in diabetes (Parhofer, 2015).

The majority of type 2 diabetes patients have dyslipidemia, which is defined by increased triglycerides, decreased HDL-C, and a preponderance of small-dense LDL particles (Wu and Parhofer 2014; Parhofer, 2015).

Biguanides, sulfonylureas, meglitinide, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter (SGLT2) inhibitors, and -glucosidase inhibitors are the most used oral diabetes drugs (Chaudhury *et al*., 2017). Hypoglycemia is related with practically all anti-diabetic medicines, with the causes including poor dose, inadequate disease, pharmacological, and nutritional guidance. Insulin delivery is frequently accompanied by injection site responses such as erythema, edoema, and pruritus. Metformin is known to cause diarrhoea, flatulence, indigestion, malabsorption syndrome, and cobalamin insufficiency. Glimepiride and glipizide have been linked to side effects such as elevated blood levels of liver enzymes. Edema, hypoglycemia, upper respiratory tract infection, heart failure, headache, fractures, myalgia, sinusitis, and pharyngitis are all documented side effects of pioglitazone (Mohiuddin *et al*., 2019).

Similarly, various medications such as PCSK9 inhibitors, statins, gemcabene, bempedoic acid, and ezetimibe are available in market to manage hyperlipidemia (Ezeh *et al*., 2021) but most are associated with significant adverse effects.

Although, wide range of safe and effective medications are present in the market to alleviate the symptoms of metabolic disorders, currently, plants also have the potential to be useful therapeutic or preventative agents for metabolic syndrome because they often contain complex mixtures of physiologically active substances

that work in different ways and may enhance one another's effectiveness (Graf *et al*., 2010). Herbal medicine has grown exponentially in recent years, and these treatments are gaining favour in both developing and developed countries due to their natural origins and lack of negative effects. Several commonly used traditional remedies are derived from medicinal plants, minerals, and organic substances (Grover *et al*., 2002). A collection of medicinal plants with established anti-diabetic and associated benefits, as well as herbal medications used in diabetes therapy, is assembled. *Allium sativum*, *Eugenia jambolana*, *Momordica charantia*, *Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Trigonella foenum graecum*, and *Withania somnifera* are among them (Modak *et al*., 2007).

Drypetes roxburghii (DR) is a member of the Euphorbiaceae family (spurge family) is widely grown medicinal plant in India. This tree contains terpenoids, mustard oils, flavonoids, tannins, alkaloids, glycosides, and phenolic compounds, and has anti-inflammatory, anti-oxidant, anti-microbial, anti-nociceptive, anti-pyretic, anti-emetic, cytotoxic, hypoglycemic, larvicidal, cytogenetic toxicity, aphrodisiac, anthelmintic, radioprotective, CNS depressant. (Dar *et al.*, 2019). Due to its rich phytochemical and medicinal profile, seeds of DR were selected for the study. Till date, the seeds of DR were not explored for its anti-hyperglycemic and anti-hyperlipidemic property. Therefore, the present study deals with the assessment of anti-hyperglycemic and anti-hyperlipidemic of DR seeds using experimental animal model.

MATERIAL AND METHODS

Chemicals: Folin-Ciocalteu reagent, sodium carbonate, gallicacid, Aluminum chloride, Quercitin, tricholoroacetic acid, 5,5'-dithiobisnitro benzoic acid (DTNB), thiobarbituric acid, pancreatic porcine α-amylase, α-glucosidase, acarbose, dithiothreitol solution, HMG CoA substrate were purchased from Sigma Aldrich (USA), Streptozotocin, was purchased from Hi-media lab pvt ltd. Atorvastatin was purchased from apollo pharmacy (East-west pharmaceutical pvt ltd.) Liver function test kit was procured from Avecon healthcare pvt ltd. Ambala, Haryana, India. Lipid profile determination kit was purchased from Tulip diagnostic pvt. Ltd. glucose, glibenclamide was purchased from Sri Pharmacare, Mumbai

Plant material: Seeds of *Drypetes roxburghii* (DR) was identified, purchased and authenticated from National Botanical Research Institute (NBRI), Lucknow, India. Reference no. NBRI/CIF/480/2015

Preparation of hydroalcoholic extract of *DR* seeds. The 200 gm of seeds of DR were divided into various fragments and dried in shade for approximately 1 month. The dried seeds were then ground into coarse powder using mechanical grinder. Afterwards, the seeds were defatted using petroleum ether using Soxhlet apparatus and process was continued till the siphon of the Soxhlet became clear. Afterwards, the marc was collected and further extracted with 70% ethanol at temperature 60-65 °C till the siphon became transparent (10-12 cycles). The obtained extract was dried in rotary evaporator. The semi-solid mass obtained was kept in refrigerator till further use.

Determination of Total Phenolic content (TPC). Folin-Ciocalteu method was used to determine the TPC in DR extract. 1.5 ml of extract (1 mg/mL) was mixed with 1.5mL of Folin-Ciocalteu reagent. After 5 minutes, the content was further mixed with 7.5mL of Na_2CO_3(200g/L). The reaction mixture was incubated for 2hours at room temperature in the dark. At 760 nm, the absorbance of the reaction mixture was recorded. The calibration curve was obtained using a standard solution of gallic acid (1mg/L to 70mg/L). The results were expressed as gallic acid equivalents (µgGAE/mg) (Yayinie *et al*., 2022; Lefahal *et al.*, 2018).

Determination of total flavonoid content (TFC). Aluminum chloride colorimetric method was used to determine the total flavonoid content in the extract. 1ml of the extract was mixed with 4mL of distilled water. Afterwards, 5ml of 2% $AlCl_3$ in methanol was added to the reaction mixture. The reaction mixture was mixed thoroughly and incubated for 10 minutes at room temperature. The calibration curve quercetin standard was prepared (1 to 30mg/mL) and absorbance was taken at 425nm. The total flavonoid content was expressed as quercetin equivalent (µgQE/mg) (Yayinie *et al*., 2022; Lefahal *et al.*, 2018).

Experimental animals: Swiss mice and wistar albino rats of either sex was purchased and housed in animal house of Siddharta Institute of Pharmacy, Dehradun, Uttarakhand, India. The animals were placed in standard condition of temperature (25 ± 2 °C), relative humidity (40-50%), and artificial lighting 12 h dark/light cycles. All the animals were given standard diet and water ad libitum. Experimentation was performed as per CPCSEA, IAEC. Protocol ID: SIP/IAEC/PCOL/17/2022.

Acute oral toxicity. Acute oral toxicity study of DR root extract was determined using Organization for Economic Co-operation and Development (OECD) guidelines 423. The rats (n=6) were administered with single dose of 2000 mg/kg BW, p.o. The animals were examined carefully after the administered of plant extract at different period of time period and afterwards examined twice for 14 days for any signs of toxicity (Schlede *et al*., 2005).

Anti-diabetic activity

Induction of diabetes. The experimental mice were fasted overnight and induced by a single intraperitoneal dose of freshly prepared streptozotocin (STZ), 150 mg/kg (prepared in 0.1 M of cold citrate buffer, pH=4.5). 30 min after administration of STZ, the mice was given

free access of water and standard diet (Alema *et al*., 2020). Thirty minutes after the STZ administration, the mice were allowed to have free access to food and water. Six hours later, the animals were fed with 5% glucose solution for the next 24 hours to prevent hypoglycemic shock and death. After four days of STZ administration, fasting blood glucose level of the mice was measured and the mice with BGL more than 200 mg/dl were included in the study (Belayneh and Birru 2018).

Experimental design: The study was carried out as per Belayneh and Birru (2018). The animal used for study was Swiss albino mice, male (as females are less sensitive to insulin and STZ). Animals were divided into various groups for all categories, as mentioned below:

(a) Evaluation of hypoglycemic activity of test extract in normoglycemic mice

(b) Evaluation of anti-hyperglycemic activity of test extract in oral glucose loaded mice.

(c) Evaluation of anti-hyperglycemic of test extract in single dose treated diabetic mice.

(d) Evaluation of anti-hyperglycemic activity of test extract in repeated dose treated diabetic mice.

Grouping and dosing

(a) In normoglycemic were divided into four experimental groups *viz*.

- Group 1: Control: The mice received distilled water (10 ml/kg, p.o)
- Group 2: positive control: The mice received Glibenclamide (5 mg/kg, BW, p.o)
- Group 3: The mice received DRL extract (100 mg/kg, BW, p.o)
- Group 4: Test group 2: The mice received DRH extract (200 mg/kg, BW, p.o)

(b) In oral glucose loaded animal model, the mice were divided into four groups viz.

- Group 1: Glucose Control: The mice received distilled water (10 ml/kg, p.o) + 2.5 g/Kg of glucose (40% w/v)
- Group 2: positive control: The mice received Glibenclamide (5 mg/kg, BW, p.o) + 2.5 g/Kg of glucose (40% w/v)
- Group 3: Test group 1: The mice received DRL extract (100 mg/kg, BW, p.o) + 2.5 g/Kg of glucose (40% w/v)
- Group 4: Test group 2: The mice received DRH extract (200 mg/kg, BW, p.o) + 2.5 g/Kg of glucose (40% w/v)

(c) In single dose treated diabetic animal model, the mice were divided into five experimental groups viz.

- Group 1: Disease Control: The mice received distilled water (10 ml/kg, p.o) + Streptozotocin (150 mg/Kg)
- Group 2: positive control: The mice received Glibenclamide (5 mg/kg, BW, p.o) + Streptozotocin (150 mg/Kg)
- Group 3: Test group 1: The mice received DRL extract (100 mg/kg, BW, p.o) + Streptozotocin (150 mg/Kg)
- Group 4: Test group 2: The mice received DRH extract (200 mg/kg, BW, p.o) + Streptozotocin (150 mg/Kg)

(d) In repeated dose treated diabetic animal model, the mice were divided into five experimental groups *viz.* and received treatment for 21 days.

- Group 1: Control: The mice received distilled water (10 ml/kg, p.o)
- Group 2: The mice received distilled water (10 ml/kg, p.o) + Streptozotocin (150 mg/Kg)
- Group 3: positive control: The mice received Glibenclamide (5 mg/kg, BW, p.o) + Streptozotocin (150 mg/Kg)
- Group 4: Test group 1: The mice received DRL extract (100 mg/kg, BW, p.o) + Streptozotocin (150 mg/Kg)
- Group 5: Test group 2: The mice received DRH extract (200 mg/kg, BW, p.o) + Streptozotocin (150 mg/Kg) (Belayneh and Birru 2018).

Determination of Blood Glucose level

Estimation of glucose level in normoglycemic mice. Overnight fasted mice were distributed in their respective group as mentioned above. The blood glucose level of the mice was measured at 0 h as baseline and then administered with their respective treatment. Afterward, the BGL was again measured at 1, 2, and 4 hours after the treatment (Belayneh and Birru 2018).

Estimation of blood glucose level in oral glucose loaded animal (Oral glucose tolerance test). In mice specifically, an overnight fast enhances insulin-dependent glucose consumption. To have a sensitive animal model for evaluating a plant extract's antihyperglycemic action, it makes sense to utilize mice for the oral glucose tolerance test. Mice were randomly assigned to treatment group as mentioned above after overnight fasting (each group containing six animals). And, then were given their respective treatment based on their grouping, as mentioned above. Thirty minutes after the treatment administration each animal received 2.5 g/kg of glucose in the form of a solution (40% w/v). Each animal's BGL was determined at 0 minutes, right before the treatment, and again at 30, 60, and 120 minutes after the oral glucose load (Belayneh and Birru 2018; Ravindra Babu, 2012).

Estimation of blood glucose level in single dose treated streptozotocin induced diabetic mice. Overnight fasted diabetic mice were distributed and given treatment as per the treatment protocol mentioned above and blood glucose level was measured right before the treatment (at 0 h) as baseline, and then at 2, 4, 6, and 8 hours after treatment (Belayneh and Birru 2018).

Estimation of blood glucose level and body weight inrepeated dose treated streptozotocin induced diabetic mice. Overnight fasted STZ-induced diabetic mice were distributed and given treatment as per their respective group mentioned above. BGL and body weight of the treated mice was measure at day 1 of the treatment (4 days after STZ injection) as a baseline glucose level and then on 7^{th} and 14^{th} day of the treatment (Belayneh and Birru 2018).

Estimation of liver enzymes. On day 15th, the serum of the mice (repeated dose treated streptozotocin induced diabetic mice) was obtained by centrifuging the blood at 3000 rpm for 15 minutes at 4°C. The collected serum was stored at 4 °C till further use. Afterwards, the serum was used for estimation of liver enzymes AST (aspartate aminotransferase), ALT (alanine transaminase), and ALP (alkaline phosphatase) (Asif *et al*., 2021).

Estimation of in-vivo oxidative marker (Estimation of GSH and MDA)

1 g of frozen liver and pancreas was homogenized in 10 ml of 0.1 M phosphate buffer (pH 7.4). Afterwards, the homogenate was centrifuged for 15 minutes at 3000 rpm. Further, the supernatant was collected for estimation of GSH and MDA. Lowry method was used to determine the concentration of protein using standard purified bovine serum albumin (Ononamadu *et al*., 2019; Lowry *et al.*, 1951).

Estimation of glutathione (GSH) level. Supernatant and tricholoroacetic acid (10%) were combined in an equal amount, and the mixture was centrifuged to separate the protein. Phosphate buffer (2 ml, pH 7.4), 5,5'-dithiobisnitro benzoic acid (DTNB), and double-distilled water (0.4 ml) were added to the resulting supernatant (0.01 ml). At 412 nm, the mixture's absorbance was measured. It was calculated as µg/g of protein.

Estimation of lipid peroxidation (measurement of MDA level). The tube containing 0.5 ml of 30% trichloroacetic acid (TCA) and 0.8 ml of thiobarbituric acid (TBA) reagent received 1 ml of the supernatant. Aluminum foil was used to cover the tubes, which were then submerged for 30 minutes in a water bath that was shaking. At 540 nm, the absorbance was measured. MDA was measured as nm/g of protein (Noeman *et al.*, 2011; Asif *et al.*, 2021).

In-vitro analysis:

Inhibition of α-amylase activity. The sample solution of pancreatic porcine α-amylase (1 U/mL of phosphate buffer, pH 6.8) and the test extracts 100 and 200 mg/mL were prepared. Further, 250 µL of sample solution of α-amylase and 100 µL of test extract and acarbose were added. The reaction mixture was incubated at 37 °C for 20 minutes followed by addition of 250 µL of 0.5 % starch. The mixture was vortexed and incubated again at 37°C for 20 minutes. Afterward, 1 ml of dinitrosalicylic acid was added to terminate the reaction. The tubes were kept in a boiling water bath for 5 minutes and cooled at room temperature and the absorbance was measured at 540 nm. 100% enzyme activity was represented by absorbance of control α-amylase at 1 U/mL without any inhibitor. Acarbose, an α-amylase inhibitor was used for comparison studies (Paun *et al*., 2020; Alema *et al*., 2020; Sekhon-Loodu and Rupasinghe 2019). The percentage inhibition of the α-amylase by the test sample was calculated as

$$\%\ \text{inhibition of}\ \alpha-\text{amylase} = \frac{(\text{Absorbance of the control} - \text{absorbance of the test sample})}{\text{Absorbance of the control}} \times 100$$

Inhibition of α-glucosidase activity. Sample solution α-glucosidase was prepared by dissolving of 0.5 U/mL of phosphate buffer, pH 6.8 and test extract 100 and 200 mg/mL were prepared. 125 µL of α-glucosidase solution, 50 µL of test sample of different concentration and 700 µL of phosphate buffer was incubated at 37 °C for 15 minutes. Afterwards, 125 µL of 5 mM p-nitrophenyl glucopyranoside was added to the reaction mixture. The reaction mixture was kept for 15 min at 37 °C. the reaction was terminated by addition of 1000 µL of Na_2CO_3 and the absorbance was measured at 405 nm. Acarbose was used for comparison studies (Paun *et al*., 2020; Alema *et al.*, 2020; Sekhon-Loodu and Rupasinghe 2019). The percentage inhibition of the α-glucosidase by the test sample was calculated as

$$\%\ \text{inhibition of}\ \alpha-\text{amylase} = \frac{(\text{Absorbance of the control} - \text{absorbance of the test sample})}{\text{Absorbance of the control}} \times 100$$

Hypolipidemic activity

Preparation of high-fat diet. High fat diet consisted of 50% corn starch, 11.25% rice powder, 01% vegetable oil, 10% egg white, 08% fish meal, 19% cellulose, 0.125% mineral complex, 0.125 % vitamin complex and 0.5% salt. (Ali *et al*., 2021)

Experimental groups. Wistar albino rats were divided into five groups with six rats in each group.

Group I: Normal control: received distilled water (10 ml/kg)

Group II: Negative control: fed with high fat diet for 30 days.

Group III: Standard control: received atorvastatin (1.2 mg/kg body weight, p.o) + high fat diet for 30 days

Group IV: Test group I: received DRL test extract (100 mg/kg, BW) + high fat diet for 30 days

Group V: Test group II: received DRH test extract (200 mg/kg, BW) + high fat diet for 30 days

Biochemical analysis. On 30th day 1 hr after drug administration, the 1 ml of blood was collected from rats via retroorbital plexus in heparinized tubes. The tubes were centrifuged for 10 min at 3000 rpm to obtain supernatant plasma. The isolated plasma was utilized for determination of total cholesterol (TC), triglyceride

(TGs), high density lipoprotein (HDL), Very low density lipoprotein (VLDL), Low density lipoprotein (LDL), triglycerides using commercial kit (span diagnostic ltd.)

Determination of body weight. The weight of rats of each experimental group was determined at day 1, day 10, day 20 and day 30 by digital weighing balance.

HMG-CoA reductase activity. On the last day of the treatment, animals were sacrificed under mild anesthesia and liver was isolated. The isolated liver was cleaned in 0.9% normal saline and homogenized in tissue homogenizer in 20% phosphate buffer, pH 7.4 and centrifuged at 10000 rpm for 10 minutes to obtain the supernatant. 50 mL of the supernatant, 260 mL of phosphate buffer pH 7.4, 42 mL of 50 mM disodium EDTA, 60 mL of 100 mM dithiothreitol solution, 50 mL of KCl solution and, 12 mL of HMG CoA substrate solution was mixed together. The reaction mixture was vortexed for a minute and incubated at 37°C for 30 minutes. Afterward, 55 mL of NADPH was added and vortexed for a minute, followed by its incubation for 10 minutes. The absorbance of the sample were taken at 340 nm (Hasimun *et al.*, 2019)

Statistical analysis. Statistical analysis was performed using graph pad prism version 9. All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance was $p<0.05$.

RESULTS

Total phenolic content. The total phenolic content of the crude extract of DR was determined using Folin-Ciocalteu reagent and was calculated from the linear regression equation of standard curve ($y = 0.0093x + 0.0622$, $R^2 = 0.9907$). The phenolic content in the crude extract was found to be 55.740 µg GAE/mg)

Total flavonoid content. The flavonoid content in the crude DR extract was determined using aluminium chloride colorimetric method and was calculated from the linear regression equation of quercitin standard curve ($y = 0.0005x + 0.0161$, $R^2 = 0.993$). The flavonoid content in the crude extract was found to be 142.87 µg QE/mg)

Acute oral toxicity studies. No sign of toxicity was observed in mice at 2000 mg/kg BW. Hence, the LD50 of the crude is considered to be more than 2000 mg/kg BW and the extract was found to be safe till 2000 mg/kg BW. To carry out the further investigation, two random doses 100 and 200 mg/kg BW was randomly selected.

Effect of DR extract on blood glucose level (BGL) in normoglycemic mice at various point of time in a day. The BGL of each treatment group in normoglycemic mice is presented in Table 1. It is clearly evident from the statistical analysis that there is no significant different in BGL among any of treatment group at 0 and 1 hr. When measured at 2 and 4 hr, BGL of glibenclamide was found to be lower than normal control ($p<0.01$). Similarly, when compared to DRL and DRH, BGL of Glibenclamide treated mice was found to be lowered ($p<0.01$) when measured at 2 and 4th h of the drug administration.

Effect of various treatments on blood glucose level in oral glucose loaded mice at different period of time in a day.

As presented in Table 2, no significant difference in baseline BGL was observed in any of the treated groups. With administration of glucose, the BGL of each treated rat was elevated and the BGL lowering effect of each treated group was evaluated. Glibenclamide treated mice lowered the glucose level compared to glucose control mice ($p<0.01$) measured at 30 min. DRL and DRH does not show any significant reduction in BGL compared to glucose control at 30 min.

BGL measurement at 60 min revealed its reduction in glibenclamide treated mice ($p<0.0001$) compared glucose control. DRH treated mice reduced BGL compared to glucose control mice ($p<0.05$). However, non-significant reduction of BGL was found in DRL treated mice compared to glucose control.

Table 1: Effect of various treatments on blood glucose level in normoglycemic mice at various point of time in a day.

Treatment groups	Blood Glucose Level (BGL) (gm/dl)			
	0 h	1 h	2 h	4 h
Control: Distilled water (10 ml/kg)	84.85±2.195	85.52±3.070	83.02±2.173	81.52 ±2.601
Glibenclamide (0.5 mg/kg, BW)	86.39 ±3.201	78.39±2.374	70.22 ±2.208[3*]	66.91±2.367[3*]
DRL (100 mg/kg, BW)	86.13±3.895	86.46±3.87	83.08±2.405[ns*,3#]	78.48±2.877 [ns*,3#]
DRH (200 mg/kg, BW)	86.20±2.535	85.70±3.036	83.70±2.405[ns*,3#]	81.70±2.342 [ns*,3#]

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance was $p<0.05$. [1] $p<0.0001$; [2] $p< 0.001$; [3] $p<0.01$; [4] $p<0.05$. * Comparison to control group; # Comparison to standard group Glibenclamide

Comparison between glibenclamide and DRL; glibenclamide and DRH treated group revealed that the BGL lowering effect of glibenclamide is highly significant ($p<0.0001$) compared to DRL and DRH treated group.

Measurement at 120 min depicted that there was a significant reduction in BGL in glibenclamide treated group and DRH treated group compared to glucose control ($p<0.0001$ and $p<0.01$, respectively). Comparison between glibenclamide and DRL; glibenclamide and DRH treated group revealed that the BGL lowering effect of glibenclamide was significant compared to DRL ($p<0.01$) compared to DRL treated groups. No significant difference in BGL was observed between glibenclamide and DRH, which depicts that the effect of DRH was comparable to that of standard glibenclamide. Data is presented in Table 2.

Table 2: Effect of various treatments on blood glucose level in oral glucose loaded mice at different point of time in a day.

Treatment groups	Blood Glucose Level (BGL) (gm/dl)			
	0 min	30 min	60 min	120 min
Glucose Control: Distilled water (10 ml/kg)	86.02±2.121	217.16±8.715	184.13±9.862	118.186±1.579
Glibenclamide (0.5 mg/kg, BW)	87.226±3.844	$176.39±6.340^{3*}$	$86.893±3.466^{1*}$	$84.07±3.263^{1*}$
DRL (100 mg/kg, BW)	88.296±1.983	$204.70±9.20^{ns*\#}$	$169.58±3.641^{ns*1\#}$	$109.74±7.144^{ns*,3\#}$
DRH (200 mg/kg, BW)	85.04±1.983	$189.373±4.384^{ns*\#}$	$152.04±6.432^{4*1\#}$	$93.20±2.472^{3*,\ ns\#}$

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance was $p<0.05$. [1] $p<0.0001$; [2] $p< 0.001$; [3] $p<0.01$; [4] $p<0.05$. * Comparison to control group; # Comparison to standard group Glibenclamide.

Effect of various treatments in single dose on blood glucose level in diabetic mice at various point of time in a day. Diabetic mice were treated with their respective treatment and BGL was measured at different point of time in a day. At 0 h, i.e., right after the treatment the BGL of each mice in each treatment group was measured and no significant difference in BGL was observed between any of the treatment group.

When measured at 2 h, the BGL level of glibenclamide and DRH treated group was significantly lowered compared to diabetic control ($p<0.0001$) and no significant difference is observed in BGL level between DRL treated group and diabetic control group. BGL of DRL and DRH was compared with glibenclamide treated group and no significant different was observed between the glibenclamide treated and DRH treated group which signifies that the BGL lowering potential of DRH was comparable to standard glibenclamide.

At 4 h, significant difference in BGL was observed when glibenclamide, DRL, and DRH treated mice were compared with diabetic control ($p<0.0001$). Comparison between glibenclamide and other treatment group (DRL and DRH) revealed a significant difference between in BGL level between glibenclamide and DRL ($p<0.0001$) and glibenclamide and DRH ($p<0.05$).

At 6 and 8 h, significant difference was observed in BGL when glibenclamide, DRL, and DRH treated mice were compared with diabetic control ($p<0.0001$). Significant difference in BGL was observed when glibenclamide treated group was compared with DRL treated group ($p<0.0001$). No significant difference was observed when glibenclamide treated group was compared with DRH treated group, which signifies that the BGL lowering effect of DRH was similar to standard glibenclamide. The results revealed the anti-hyperglycemic activity of the extract and most significantly by DRH 200 mg/kg). Data is presented in Table 3.

Table 3: Effect of various treatments in single dose on blood glucose level in diabetic mice at various point of time in a day.

Treatment	Blood Glucose Level (BGL) (gm/dl)				
	0 h	2 h	4 h	6 h	8h
Diabetic control	397.38±3.185	398.34±4.709	398.17±5.182	396.61±4.199	392.84±5.827
Glibenclamide (0.5 mg/kg, BW)	399.44±4.722	$313.16±5.735^{1*}$	$215.39±9.928^{1*}$	$185.97±9.71^{1*}$	$161.5±4.814^{1*}$
DRL (100 mg/kg, BW)	392.77± 51.914	$374.44±10.276^{ns*,\ 1\#}$	$298.7±2.557^{1*,\ 1\#}$	$249.74±3.741^{1*,1\#}$	$212.89±4.681^{1*,1\#}$
DRH (200 mg/kg, BW)	394.5± 4.681	$323.81±6.314^{1*,\ ns\#}$	$242.5±6.474^{1*,\ 4\#}$	$198.76±2.977^{1*,\ ns\#}$	$180.76±4.108^{1*,\ ns\#}$

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance was $p<0.05$. [1] $p<0.0001$; [2] $p< 0.001$; [3] $p<0.01$; [4] $p<0.05$. * Comparison to diabetic control group; # Comparison to standard group Glibenclamide

Effect of various treatments in repeated dose on blood glucose level and percent reduction in BGL from baseline level in diabetic mice at various days during a treatment period of 14 days. The result of repeated administration of various treatment on BGL is presented in Table 4. The baseline BGL was all the treatment group was found to be significantly higher than normal control group ($P<0.0001$) and no significant difference in BGL was observed between diabetic control group and other treatment groups (glibenclamide, DRL, DRH).

The BGL level was later observed at 7 and 14th day of treatment. On 7th day, BGL level of diabetic control mice, glibenclamide, DRL, and DRH treated group was found to be significantly higher than control group ($p<0.0001$, $p<0.05$, $p<0.0001$, $p<0.01$, respectively). When compared to diabetic control group, BGL was found to be reduced significantly in glibenclamide, DRL and DRH treated group ($p<0.0001$). Comparison between standard group glibenclamide and extract treated group (DRL and DRH) revealed, significant difference in BGL reduction in glibenclamide compared to DRL ($p<0.05$) and non-significant difference in BGL between glibenclamide and DRH treated group which signifies that effect of DRH 200 mg/kg BW was equal to standard glibenclamide.

Similarly, on 14th day of treatment, BGL of diabetic control group mice, glibenclamide treated mice, DRL treated mice and DRH treated mice was found to be significantly higher compared normal control group ($p<0.0001$, $p<0.05$, $p<0.0001$, $p<0.001$). When compared to diabetic control, glibenclamide, DRL and DRH treated groups significantly lowered the BGL ($p<0.0001$). When compared with glibenclamide, a significant different in BGL was found between glibenclamide and DRL ($p<0.01$). No significant difference in BGL between glibenclamide and DRH was observed which signifies that the effect of DRH 200 mg/kg BW. The results depict that DRL, DRH, and glibenclamide showed significant antihyperglycemic activity and the effect of DRH 200 mg/kg was as potent as glibenclamide.

Percent reduction in BGL from baseline was also determined. Among all the treatments, glibenclamide showed highest percent reduction in BGL by 61.65% (7th day) and 67.78% (14th day), followed by DRH 200 mg/kg which is 55.97 (7th day) and 63.87 (14th day), followed DRL by 45.68 (7th day) and 63.87 (14th day). The result of the above parameters suggests that DR extract showed potent antihyperglycemic activity and this effect was more prominent with dose 200 mg/kg BW.

Table 4: Effect of various treatments in repeated dose on blood glucose level and percent reduction in BGL from baseline level in diabetic mice at various days during a treatment period of 14 days.

Treatments	Blood Glucose Level (BGL) (gm/dl)			Percent reduction in BGL from baseline	
	Baseline	7 day	14 day	7 day	14 day
Control	85.72±2.15	86.02±2.81	88.02±3.69	NA	NA
Diabetic control	397.38±3.185^{1*}	399.83±5.51^{1*}	398.186±6.532^{1*}	-0.61	-0.15
Glibenclamide (0.5 mg/kg, BW)	399.44±4.722^{1*}	153.16±11.61$^{4*, 1\alpha}$	128.76±5.979 $^{4*, 1\alpha}$	61.65	67.78
DRL (100 mg/kg, BW)	392.77±5.914^{1*}	213.37±10.225$^{1*,1\alpha,4\#}$	182.18±14.936 $^{1*,1\alpha, 2\#}$	45.68	53.66
DRH (200 mg/kg, BW)	394.5±4.681^{1*}	173.72±24.070$^{2*,1\alpha\ ns\#}$	142.5±6.47, $^{3*, 1\alpha, ns\#}$	55.97	63.87

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance was $p<0.05$. [1] $p<0.0001$; [2]$p< 0.001$; [3]$p<0.01$; [4]$p<0.05$. * Comparison to control group; # Comparison to standard group Glibenclamide; α com

Effect of various treatments in repeated dose on body weight in diabetic mice at various days during a treatment period of 14 days. Body weight of mice in each experimental group were determined before the induction of diabetes, than after four days of induction of diabetes (baseline), then on 7th and 14th day of the treatment. No significant difference in body weight was observed among various treatment groups before the induction of diabetes. The difference in baseline body weight among control and other treatment groups (Glibenclamide, DRL and DRH treated mice) was found to be insignificant, whereas a significant difference in body weight of control mice and diabetic control mice was observed ($p<0.05$) (the body weight of diabetic control mice was found to be less. Body weight measurement on 7th day of treatment revealed a significant lowered body weight of diabetic control mice, DRL and DRH treated mice ($p<0.0001$, $p<0.001$, and $p<0.01$ respectively. However, the difference in body weight of control mice and glibenclamide treated mice was found to be insignificant. When compared to diabetic control group, a non-significant difference in body weight was found in glibenclamide, DRL, and DRH treated groups.

On 14th day of the treatment, measurement of body weight revealed a significantly lowered body weight of diabetic control mice, DRL and DRH treated mice ($p<0.0001$, $p<0.001$, and $p<0.01$) when compared to normal control. The difference in body weight between control and glibenclamide treated mice was found to be insignificant. When compared to diabetic control all the treatment group mice i.e. glibenclamide, DRL, and DRH

treated mice showed significantly lowered body weight ($p<0.0001$, $p<0.01$, and $p<0.001$ respectively). Comparison to glibenclamide treated mice, the lowering of body weight by DRL and DRH treated mice was insignificant. Data is presented in table 5.

The result presents that there was substantial loss of body weight of all the mice there was induced with diabetes and treatment with glibenclamide, DRL and DRH increased the body weight of mice to a significant level. Moreover, the effect of DRL and DRH was found to be similar to standard glibenclamide.

Table 5: Effect of various treatments in repeated dose on body weight in diabetic mice at various days during a treatment period of 14 days.

Treatments	Body weight (gm)			
	Before induction of diabetes	Baseline	7th day	14th day
Control	26.156±0.583	27.143±0.742	27.31±0.535	27.27±0.567
Diabetic control	25.82±0.687	24.591±0.418[4*]	23.08±0.400[1*]	20.76±0.357[1*]
Glibenclamide (0.5 mg/kg, BW)	26.42±0.553	25.705±0.419[ns*α]	25.17±0.522[ns*α]	25.65±0.361[ns*1α]
DRL (100 mg/kg, BW)	26.335±0.588	25.42±0.433 [ns*α#]	24.11±0.486[3*nsα#]	24.35±0.543[3*,2α,ns#]
DRH (200 mg/kg, BW)	26.155±0.761	24.693±0.819 [ns*α#]	23.311±0.691[2* nsα#]	23.81±0.723[2*,3α, ns#]

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance were $p<0.05$. [1] $p<0.0001$; [2]$p< 0.001$; [3]$p<0.01$; [4]$p<0.05$. * Comparison to control group; # Comparison to standard group Glibenclamide.

Effect of various treatments in repeated dose on liver enzymes in diabetic mice at the end of the treatment. On the last day of the treatment, liver enzymes (AST, ALT, and ALP) were estimated. All the liver enzymes (AST, ALT, and ALP) were significantly elevated in diabetic control mice ($p<0.0001$) compared to control group mice.

AST level of glibenclamide, DRL and DRH treated mice was significantly reduced ($p<0.0001$) when compared to diabetic control group. The AST level of DRL and DRH treated mice was similar to that of glibenclamide treated mice, as no significant difference in AST value was observed among them.

ALT level of glibenclamide, DRL and DRH treated mice was significantly reduced ($p<0.0001$) when compared to diabetic control group. The AST level of DRH treated mice was found to be similar to that of glibenclamide treated group, as no significant difference was observed between them. However, a significant difference in AST reduction was found between glibenclamide and DRL treated group ($p<0.01$), with more pronounced effect of former in lowering ALT compared to later.

ALP level of glibenclamide, DRL and DRH treated mice was significantly reduced ($p<0.0001$) when compared to diabetic control group. The ALP level of DRH treated mice was found to be similar to that of glibenclamide treated group, as no significant difference was observed between them. However, a significant difference in ALP reduction was found between glibenclamide and DRL treated group ($p<0.0001$), with more pronounced effect of former in lowering ALT compared to later. Data is presented in table 6.

The result depicts that there was significant elevation of various liver enzymes in diabetic control mice and treatment with glibenclamide, DRL, and DRH brought the liver enzyme to normal level depicting its hepatoprotective function. The effect of DRH was found to be similar to glibenclamide treated group.

Table 6: Effect of various treatments in repeated dose on liver enzymes in diabetic mice at the end of the treatment.

Treatments	Liver enzymes		
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	93.105±2.051	42.606±2.535	125.22±1.957
Diabetic control	151.22±2.914[1*]	96.54±3.00[1*]	254.59±6.452[1*]
Glibenclamide (0.5 mg/kg, BW)	88.70±2.081[ns*1α]	41.105±1.190[ns*1α]	123.55±2.772[ns*,1α]
DRL (100 mg/kg, BW)	97.05±3.893[ns*,1αns#]	59.26±3.114[2*,1α2#]	195.75±4.325[1*, 1α,1#]
DRH (200 mg/kg, BW)	91.89±2.406 [ns*, 1α, ns#]	46.241±1.951[ns*, 1α,ns#]	133.38±5.945[ns*, 1α,ns#]

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance was $p<0.05$. [1] $p<0.0001$; [2]$p< 0.001$; [3]$p<0.01$; [4]$p<0.05$. * Comparison to control group; # Comparison to standard group Glibenclamide; **α** compared to diabetic control

Effect of various treatments in repeated dose on GSH and MDA level in diabetic mice at the end of the treatment. At the end of the treatment, GSH and MDA level in liver and pancreas of the mice were determined. The liver's and pancreas's GSH level were significantly reduced in diabetic control mice ($p<0.0001$) compared to

control group mice. When compared to diabetic control, glibenclamide, DRL and DRH treated mice showed elevated liver's GSH level ($p<0.0001$, $p<0.01$, $p<0.0001$ respectively). A significant difference was observed in liver GSH level of glibenclamide treated mice and DRL treated mice ($p<0.001$); and glibenclamide treated mice and DRL treated mice ($p<0.01$). Pancreas GSH level of glibenclamide, DRL and DRH treated mice was significantly elevated ($p<0.0001$) when compared to negative control and the effect of DRL and DRH was similar to that of glibenclamide, as no significant difference in pancreas's GSH level was observed between glibenclamide and DRL treated mice; and between glibenclamide and DRH treated mice. Liver and pancreas MDA level were found to be elevated in diabetic control mice ($p<0.0001$) as compared to normal control mice. Liver and pancreas MDA level of glibenclamide, DRL, and DRH treated mice was found to be significantly reduced ($p<0.0001$) in comparison to diabetic control. Also, the effect of DRL and DRH was found to be similar to that of glibenclamide, as no significant difference in liver and pancreas's MDA level was observed between glibenclamide and DRL treated mice; and between glibenclamide and DRH treated mice. Elevation in GSH level and reduction in MDA level by DR extract (DRL and DRH) depicts that the extract exhibits anti-oxidant potential. The effect of DR extract was found to comparable to that of standard glibenclamide.

Table 7: Effect of various treatments in repeated dose on GSH and MDA level in diabetic mice at the end of the treatment.

Treatments	GSH (mg/gm of protein)		MDA (nm/g of protein)	
	Liver	Pancreas	Liver	Pancreas
Control	22.07±0.569	23.93±0.954	9.87±1.58	8.908±0.982
Diabetic control	8.97±0.693^{1*}	7.55±0.802^{1*}	28.52±0.901^{1*}	28.39±0.892^{1*}
Glibenclamide (0.5 mg/kg, BW)	22.25±1.077ns$^{*, 1\alpha}$	23.40±1.345$^{ns*, 1\alpha}$	14.44±0.893$^{ns*, 1\alpha}$	14.31±1.196$^{4*,1\alpha}$
DRL (100 mg/kg, BW)	15.38±0.872$^{2*, 3\alpha\ 2\#}$	21.68±0.889$^{ns*, 1\alpha, ns\#}$	14.87 ±1.196$^{4*, 1\alpha ns\#}$	18.57±1.145$^{1*, 1\alpha, ns\#}$
DRH (200 mg/kg, BW)	16.86±1.471$^{3*, 1\alpha, 3\#}$	22.85±0.99$^{ns*, 1\alpha, ns\#}$	13.22±1.117$^{ns*, 1\alpha ns\#}$	13.22±1.185$^{ns*,1\alpha, ns\#}$

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance was $p<0.05$. [1] $p<0.0001$; [2]$p< 0.001$; [3]$p<0.01$; [4]$p<0.05$. * Comparison to control group; # Comparison to standard group Glibenclamide; **α** compared to diabetic control.

In-vitro anti-diabetic activity. As shown in Fig. 1, acarbose shown the maximum inhibition of α-amylase and α-glucosidase, i.e. 82.95±2.081 and 73.28±0.94 respectively, followed by DRH 76.02± 0.648 % of α-amylase and 66.78± 0.612 % α-glucosidase, followed by DRL 68.3± 1.42 % α-amylase and 57.75 ± 0.535 % of α-glucosidase.

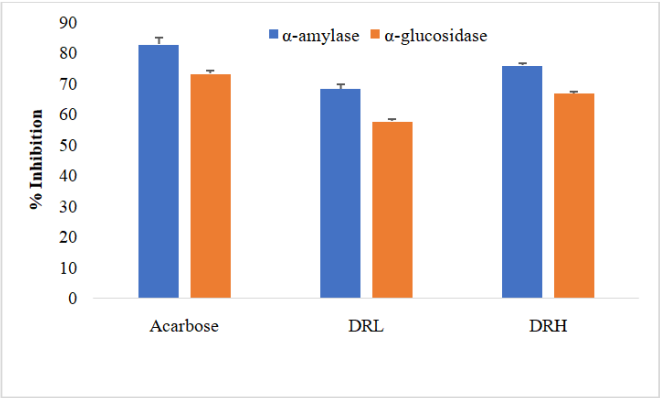

Fig. 1. Effect of various treatment on inhibition of α-amylase and α-glucosidase.

Anti-hyperlipidaemic activity

Effect of various treatments on body weight of high fat diet induced hyperlipidemic rats over a period of 30 days. Body weight of each rat of each experimental group were measured on day 1, 10, 20, and 30 of the treatment. On day 1, the body weight of rat in each experimental group were compared and non-significant differences in body weight was observed among each treatment group. After 10 days, the body weight was again measured, and significant increase was found between control group and HFD control group ($p<0.0001$), atorvastatin group ($p<0.05$), DRL ($p<0.0001$) and DRH ($p<0.0001$) treated group. Comparison of Atorvastatin, DRL and DRH treated group with HFD control group revealed the significant decrease in the body weight ($p<0.0001$). DRL and DRH

were further compared with standard atorvastatin, where significant difference in body weight between atorvastatin and DRL ($p<0.001$); and atorvastatin and DRH ($p<0.01$). The body weight lowering effect of atorvastatin was more pronounced then DRL and DRH.

Measurement of body weight on Day 20 showed significant increase in body weight of HFD control rat, atorvastatin, DRL, and DRH treated rats ($p<0.0001$, $p<0.001$, $P<0.0001$, and $p<0.0001$ respectively) compared to control group. When compared with HFD control group, the body weight of atorvastatin and DRH treated rats were significantly reduced ($p< 0.001$ and $p<0.01$ respectively), whereas no significant reduction in body weight was found between HFD control group rat and DRL treated rats. Comparison of DRL treated rat with atorvastatin treated rat, a significant difference was found in body weight ($p<0.0001$), whereas comparison of DRH treated rat with atorvastatin treated rat had no significant difference in body weight which signifies that the effect of DRH was comparable to that of atorvastatin. Similar pattern was observed after 30 day of treatment.

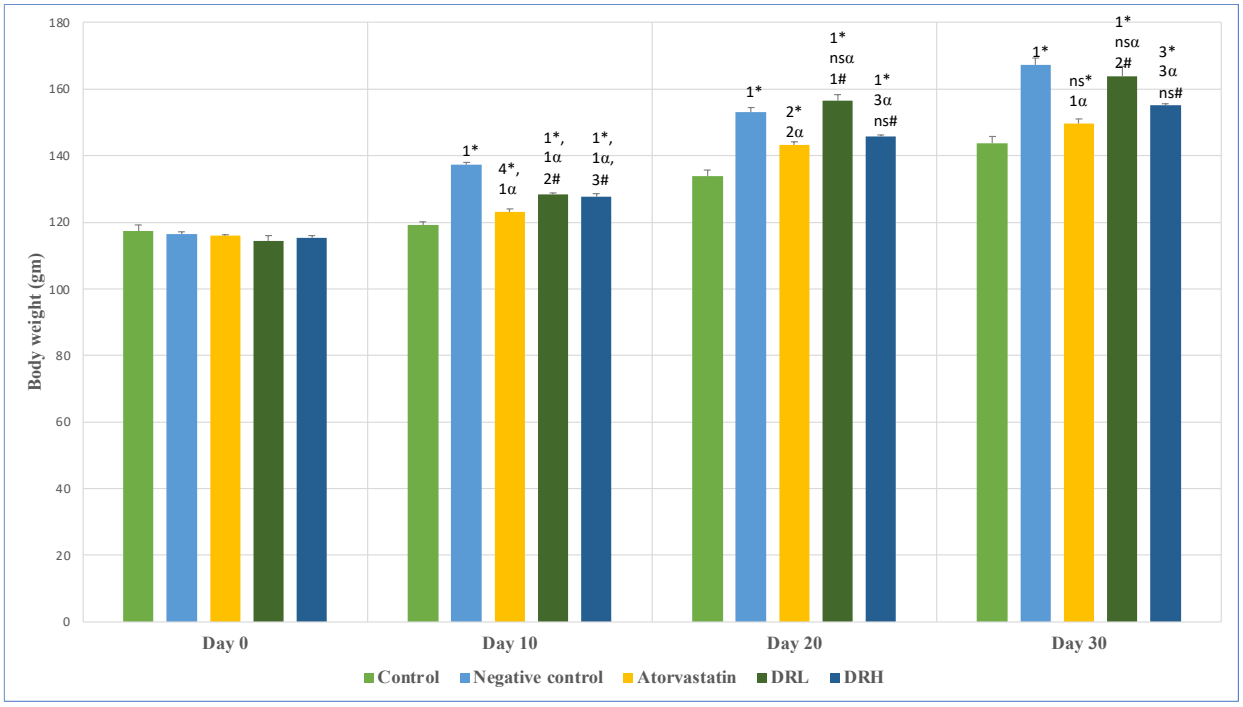

Fig. 2. Effect of various treatments on body weight of high fat diet induced hyperlipidemic rats over a period of 30 days.

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by dunnett's test of multiple comparisons. The criteria of statistical significance was $p<0.05$. [1] $p<0.0001$; [2] $p< 0.001$; [3] $p<0.01$; [4] $p<0.05$. * Comparison to control group; $^{\alpha}$ Comparison to HFD control; # comparison to atorvastatin

Effect of various treatments in lipid profile of high fat diet induced hyperlipidemia in rats. After the last day of treatment, the plasma of the mice of all treatment groups was isolated and analyzed for its lipid profile. Data is presented in Table 8.

LDL of HFD control rats was found to be significantly higher ($p<0.0001$) compared to control group. Treatment with atorvastatin, DRH and DRL significantly lowered the LDL level in rats ($p<0.0001$, $p<0.0001$, and $p<0.01$ respectively) compared HFD control rats. The effect of DRL in lowering of LDL was significantly different compared to standard atorvastatin ($p<0.05$), with later having the more pronounced effect compared to the former, whereas, non-significant difference in LDL level was found between DRH and atorvastatin which signifies that the effect of DRH was comparable to that of standard atorvastatin. Also, non-significant difference in LDL level was found between control and atorvastatin group; and control and DRH treated group. The result signifies that DR extract was able to lower the LDL level and the effect of DRH (200 mg/kg) was as significant as standard atorvastatin.

VLDL of HFD control rats was found to be significantly higher ($p<0.001$) compared to control group. Treatment with atorvastatin, DRH and DRL significantly lowered the VLDL level in rats ($p<0.001$, $p<0.001$, and $p<0.01$ respectively) compared HFD control rats. Non-significant difference in VLDL level was found between DRH and atorvastatin; and DRL and atorvastatin which signifies that the effect of DR extract at both the doses 100 and 200 mg/kg were comparable to that of standard atorvastatin. Also, non-significant difference in VLDL level was found between control and atorvastatin group; control and DRH treated group; and control and DRL

treated group. The result signifies that DR extract was able to lower the VLDL level and the effect was as significant as standard atorvastatin.

Significant reduction in HDL level was found in HFD control group rats ($p<0.001$) compared to normal control group rats. Treatment with atorvastatin and DRH significantly enhanced the HDL level in rats ($p<0.001$, and $p<0.05$, respectively) compared HFD control rats. However, treatment with DRL does not enhance the HDL level compared to HFD control group rats. The effect of DRL in enhancing of HDL was significantly different compared to standard atorvastatin ($p<0.05$), with later having the more pronounced effect compared to the former, whereas, non-significant difference in HDL level was found between DRH and atorvastatin which signifies that the effect of DRH was comparable to that of standard atorvastatin. Also, non-significant difference in HDL level was found between control and atorvastatin group; and control and DRH treated group. The result signifies that DR extract was able to enhance the HDL level and the effect of DRH (200 mg/kg) was as significant as standard atorvastatin.

Similarly, HFD control rats showed significantly higher level of TC and TG when compared to normal control group ($p<0.0001$). Atorvastatin, DRH, and DRL treated rats showed significantly lowered TC ($p<0.0001$, $p<0.001$, and $p<0.05$) compared to HFD control rats. Comparison between DR extract (DRL and DRH) with standard drug (atorvastatin) revealed a significant difference in TC level was observed between atorvastatin and DRL treated rat ($p<0.01$) and a non-significant difference in TC value was observed between atorvastatin and DRH, which signifies that the effect of DRH 200 mg/kg was comparable to that of standard atorvastatin.

As for TG, Atorvastatin, DRH, and DRL treated rats showed significantly lowered TG ($p<0.0001$, $p<0.0001$, and $p<0.01$) compared to HFD control rats. Comparison between DR extract (DRL and DRH) with standard drug (atorvastatin) revealed a significant difference in TG level was observed between atorvastatin and DRL treated rat ($p<0.0001$); and atorvastatin and DRH treated rat ($p<0.01$).

The result signifies that the DR extract at both the doses were able to normalize the lipid profile of high fat diet induced hyperlipidemia in rats. The effect of DR extract at dose 200 mg/kg was found to be equivalent to that of standard atorvastatin, as in most of the lipid parameter the difference in the values were found to be non-significant.

Table 8: Effect of various treatments in lipid profile of high fat diet induced hyper lipidemia in rats.

Treatments	Lipid profile (gm/L)				
	LDL	VLDL	HDL	TC	TG
Control	2.72±0.507	1.385±0.13	4.86±0.141	9.18±0.599	7.215±0.327
HFD control	7.075±0.465[1*]	3.193±0.353[2*]	3.211±0.396[3*]	13.673±0.504[1*]	13.07±0.515[1*]
Atorvastatin	2.963±0.262 [ns*,1α]	1.645±0.240[ns*, 3α]	4.95±0.350[ns*,3α]	8.25±0.360[ns*,1α]	7.055±0.320[ns*,1α]
DRL	4.89±0.557 [4*,3α, 4#]	2.035±0.257[ns*,4α, ns#]	3.62±0.180[4*, nsα,4#]	11.19±0.233[ns*,4α,3#]	11.0±0.36 [1*, 3α, 1#]
DRH	3.4±0.1861#, [ns*, 1α, ns#]	1.79±0.240[ns*,3α, ns#]	4.25±0.330[ns*, 4α, ns#]	10.034±0.759[ns*,2α, ns#]	9.005±0.203[4*, 1α,3#]

All data has been expressed as Mean ± SEM. Data has been analyzed using ANOVA followed by Dunnett's test of multiple comparisons. The criteria of statistical significance were $p<0.05$. [1] $p<0.0001$; [2] $p< 0.001$; [3] $p<0.01$; [4] $p<0.05$. * Comparison to control group; # Comparison to standard atorvastatin; ᵅ Comparison to HFD control

Effect of various treatments on HMG-CoA reductase activity. As shown in figure 3, atorvastatin a standard HMG-CoA inhibitor showed inhibition of HMG-CoA reductase by 73.49 ± 2.13 %. DRL and DRH also inhibited HMG-CoA reductase by 22.24 ± 5.45 % and 46.79± 4.24 % respectively.

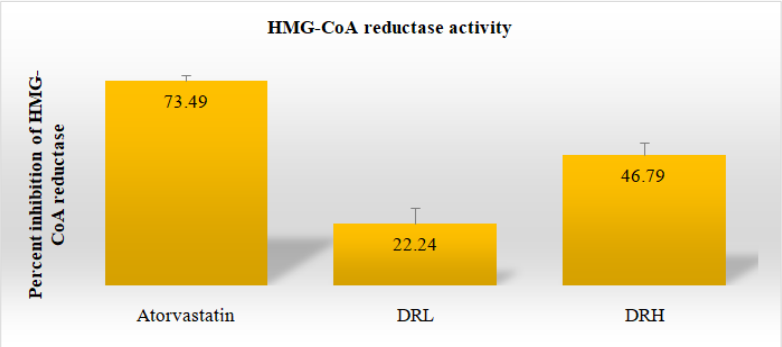

Fig. 3. Percent inhibition of HMG-CoA reductase by atorvastatin, DRL, and DRH.

DISCUSSION

In the present study, the seeds of *Drypetes roxburghii* were extracted, and their flavonoid or phenolic content was assessed. Flavonoids are a broad category of naturally occurring chemicals with various phenolic structures that are found mostly in fruits, vegetables, nuts, tea, and herbs. Past research has shown that the majority of dietary flavonoids have different medicinal benefits, including anti-diabetic properties. By blocking the -glucosidase enzyme, rutin can diminish carbohydrate absorption, kaempferol can increase glucose uptake, and luteolin can inhibit lipid formation (Praparatana *et al*., 2022).

Similarly, phenolic antioxidants offer good promise for managing type 2 diabetes by controlling hyperglycemia and accompanying macrovascular consequences, such as hypertension, and microvascular problems caused by oxidative damage to cells. High phenolic antioxidant activity shows that some species have phenolic chemicals that might be advantageous to human health if incorporated into food designs for a healthy diet (Lin *et al*., 2016).

As a substantial amount of flavonoid and phenolic compounds was quantitatively estimated, the extract was evaluated for anti-diabetic and anti-hyperlipidemic activity. For evaluation of anti-diabetic activity, the effect of the extract on the glucose level of normoglycemic mice, oral glucose loaded mice, single dose treated diabetic mice, and repeated-dose treated diabetic mice was seen.

The glucose tolerance test (GTT) examines the body's ability to eliminate a glucose load. It is used to detect pathological alterations in glucose metabolism that are linked to diabetes and metabolic illnesses. Before administering a glucose solution, the blood glucose levels of animals are measured after fasting. Blood glucose concentrations are then tested over a period of 2 hours (King, 2020).

Based on the GTT, mice with BGL greater than 200 gm/dL were further included in the study. First, diabetes was induced by streptozotocin. Streptozotocin is frequently employed for generating diabetes in laboratory animals. DNA is alkylated when streptozotocin enters the B cell via the glucose transporter (GLUT2). DNA damage triggers poly ADP-ribosylation activation, a mechanism that is more significant for streptozotocin's diabetogenicity than DNA damage itself. Poly ADP-ribosylation depletes NAD+ and ATP within the cell. Increased ATP dephosphorylation following treatment with streptozotocin provides a substrate for xanthine oxidase, leading to the production of superoxide radicals. Moreover, hydrogen peroxide and hydroxyl radicals are produced. In addition, streptozotocin releases hazardous levels of nitric oxide, which inhibits aconitase action and contributes to DNA damage. As a result of the activity of streptozotocin, B cells are destroyed by necrosis (Lenzen *et al*., 2007).

After the induction of diabetes, the extract was administered in a single dose as well as in repeated dose over the period of 14 days to evaluate its anti-diabetic potential. The extract was found to have potent antihyperglycemic activity as it lowered the BGL over a period of time, both in single and repeated-dose study. More over the effect of the extract at 200 mg/kg BW was found to be equivalent to standard glibenclamide. The liver is an essential metabolic organ that plays a crucial role in regulating the glucose homeostasis. The liver dysfunction indicators alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glutamyltransferase (GGT) have been demonstrated to be a reliable indication of liver health and are implicated in hepatic insulin resistance. (Islam *et al*., 2020). A greater rate of abnormal liver function tests has been related with those with Type 2 diabetes mellitus compared to those without T2DM (Mandal *et al*., 2018). Several studies have repeatedly linked elevated liver enzymes blood levels to an increased risk of acquiring type 2 diabetes (Noordam *et al*., 2017). Therefore, liver enzymes such as AST, ALT, and ALP were determined. The extract was able to bring down the liver enzymes to the normal level.

In diabetes mellitus, mitochondria are the primary contributors of oxidative stress. During oxidative metabolism, in mitochondria, a portion of the consumed oxygen is converted to water, and the remaining oxygen is converted to oxygen free radical (O•), an essential ROS that is changed to other reactive species (RS) such as ONOO, OH, and H_2O_2. ROS and RNS, which affect insulin signaling in two ways. On one hand, ROS/RNS are formed in response to insulin in order to carry out its complete physiological function. On the other hand, ROS and RNS have a detrimental effect on insulin signaling, leading to insulin resistance, which acts as a risk factor for type 2 diabetes (Asmat *et al*., 2016). Therefore, GSH and MDA level in liver and pancreas of diabetic mice and repeated-dose treated diabetic mice were determined. The extract was able to elevate GSH level and lower MDA level. As flavonoid and phenolic compound are known anti-oxidants, their presence in the plant extract serves one of the reasons of its antioxidant property.

The inhibition of alpha-glucosidase and alpha-amylase, enzymes involved in the digestion of carbohydrates, can greatly minimize the postprandial rise in the blood glucose and is consequently a key technique for managing blood glucose levels in type 2 diabetic and borderline individuals. There is a resurgence of interest in plant-based medications and functional meals that modulate physiological effects for the treatment and prevention of diabetes and obesity. The plant world provides a vast area in which there is wide scopeto look for natural, effective oral hypoglycemic medicines with minimal or no adverse effects. Hence, natural alpha-

glucosidase and alpha-amylase inhibitors derived from plants provide a promising method for the management of hyperglycemia (Tundis *et al*., 2010). Therefore, alpha-glucosidase and alpha-amylase inhibitory activity of extract was checked using acarbose as a standard. The extract significantly inhibited alpha-glucosidase and alpha-amylase (*in-vitro*).

The results of the anti-diabetic study revealed that the extract, particularly at dose 200 mg/kg, possessed potent anti-hyperglycemic activity which may be attributed to the presence of flavonoid and phenolic compound in it.

Hyperlipidemia, a key modifiable risk factor for atherosclerosis and cardiovascular disease, is a set of metabolic diseases defined by high lipid levels. Cholesterol, cholesterol esters, phospholipids, and triglycerides are examples of lipids. High LDL levels are associated with the onset of atherosclerosis (Karam *et al*., 2018). For decades, rats have been fed fat-rich diets to simulate obesity, dyslipidemia, and insulin resistance. It has been noticed that problems caused by a diet rich in fat closely match the human metabolic syndrome, and this may extend to cardiovascular issues. Essentially, all laboratory rodent species are susceptible to metabolic disturbances under these feeding conditions (Buettner *et al.,* 2006).

Therefore, based on the literature, a high fat diet was prepared and fed to the rats for 30 days. From day 1 to day 30, the body weight of the rats with and without treatment was estimated. After the last administration of treatment, the lipid profile was determined.

A high-fat diet administered to an untreated rat showed higher levels of LDL, VLDL, TC, and TG and a lower level of HDL. Whereas, treatment with standard atorvastatin and DR extract lowered the LDL, VLDL, TC, and TG levels and enhanced the HDL level. Dyslipidemia is also one of the most significant risk factors for illnesses such as atherosclerosis, which promotes the accumulation of oxidised LDL in the artery wall. Oxidized LDL has a significant role in the onset and progression of the cardiovascular dysfunction associated with atherosclerosis; therefore, reducing oxidative stress is as essential as treating dyslipidaemia. Oxidative modification of LDL is regarded as the initial step in the transformation of LDL into an atherogenic state. Serum levels of TC, TG, and HDL were significantly decreased in rats treated with atorvastatin. HDL is inversely proportional to total body cholesterol, and a decrease in plasma HDL content may hasten the development of atherosclerosis, which leads to ischemic heart disease, by impeding the removal of cholesterol from the artery wall. HDL is primarily responsible for transporting cholesterol, a process in which excess tissue cholesterol is taken up and processed by HDL particles before being transported to the liver for processing. Hence, an increase in HDL can lead to a reduced risk of atherosclerosis. Lipid peroxidation is produced by an imbalance in lipid metabolism, and it is generally known that high plasma levels of LDL and VLDL are coronary heart disease risk factors. The direct relationship between LDL level and atherosclerosis, as well as the reversibility of atherosclerosis-related pathological outcomes by reducing serum LDL, has been established previousl (Ali *et al*., 2021).

HMG-CoA reductase is the rate-limiting enzyme in cholesterol production, and it is controlled by sterols and non-sterol metabolites generated from mevalonate, the result of the process catalysed by reductase. Usually, cholesterol produced from the internalisation and breakdown of low density lipoprotein (LDL) via the LDL receptor suppresses this enzyme in mammalian cells. Competitive inhibitors of the reductase promote the development of LDL receptors in the liver, which enhances plasma LDL catabolism and decreases plasma cholesterol levels, a major predictor of atherosclerosis. (*HMGCR 3-hydroxy-3-methylglutaryl-CoA Reductase [Homo Sapiens (Human)] - Gene - NCBI*, 2018). Therefore, HMG-CoA reductase inhibition was checked, and DR extract was found to be a potent HMG-CoA reductase inhibitor.

From a study it is clear that the majority of polyphenolic rings found in plants may provide steric hindrance to NADP+ binding. Several of the identified polyphenols occupy the HMG-CoA binding site and compete with the enzyme for substrate binding (Islam *et al*., 2015). Many dietary flavonoids have been shown in vitro to reduce LDL levels and block oxidative modification, making them potential inhibitors of LDL oxidation and subsequent atherogenesis. Flavonoids reduce LDL oxidation by many mechanisms that work together: (a) by directly scavenging some radical species and thus acting as chain-breaking antioxidants; (b) by replenishing the limited supply of endogenous chain-breaking antioxidants such as -tocopherol and donating hydrogen atoms to the oxidised forms; and (c) by chelating divalent pro-oxidant transition metals such as iron and copper and thus preventing free radical formation (Unnikrishnan *et al*., 2014).

Therefore, the flavonoids and phenolic compounds present in the extract may be responsible for anti-hyperlipidemic activity of the extract.

CONCLUSIONS

From the above finding, it can be concluded that *Drypetes roxburghii* root extract was found to have potent anti-hyperglycemic and anti-hyperlipidemic activity, which may be attributed to the presence of flavonoids and phenolic compounds (potent antioxidants) in it.

FUTURE SCOPE

In future, the crude extract of *Drypetes roxburghii* root may be explored for the type of flavonoids and phenolic compounds present in it. The mechanism by which *Drypetes roxburghii* root is acting as ananti-hyperglycemic and anti-hyperlipidemic may also be exploited.

REFERENCES

Alema, N. M., Periasamy, G., Sibhat, G. G., Tekulu, G. H. and Hiben, M. G. (2020). Antidiabetic Activity of Extracts of Terminalia brownie Fresen. Stem Bark in Mice. *Journal of Experimental Pharmacology, Volume 12*, 61–71.

Ali, S. S., Asif, M. and Khan, N. A. (2021). Anti-Atherosclerosis and Anti-Hypertensive Effects of Flavonoid Isorhamnetin Isolated from the Bark of *Cordia dichotoma* L. *Journal of Pharmaceutical Research International*, *33*(42B), 316–338.

Asif, M., Gilani, S. J., Taleuzzaman, M., Kala, C., Godara, D., Rahat, I. and Khan, N. A. (2021). GC-MS Analysis of Chemical Constituents of Hydroalcoholic Leaf Extract of Cissampelos Pareira and their Anti-Diabetic Activity. *Asian Plant Research Journal*, 36–49.

Asmat, U., Abad, K. and Ismail, K. (2016). *Diabetes mellitus* and oxidative stress-A concise review. *Saudi Pharmaceutical Journal*, *24*(5), 547-553.

Belayneh, Y. M. and Birru, E. M. (2018). Antidiabetic Activities of Hydromethanolic Leaf Extract of *Calpurnia aurea* (Ait.) Benth. Subspecies aurea (Fabaceae) in Mice. Evidence-Based Complementary and Alternative Medicine, 2018, 1–9.

Buettner, R., Parhofer, K. G., Woenckhaus, M., Wrede, C. E., Kunz-Schughart, L. A., Schölmerich, J. and Bollheimer, L. C. (2006). Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *Journal of Molecular Endocrinology*, *36*(3), 485–501.

Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N. S., Montales, M. T., Kuriakose, K., Sasapu, A., Beebe, A., Patil, N., Musham, C. K., Lohani, G. P. and Mirza, W. (2017). Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. *Frontiers in endocrinology*, *8*, 6.

Dar, P., Faisal, M., Dar, A. and Waqas, U. (2019). Journey Describing Biological Activities and Chemical Constituents in the Leaves, Stem Bark and Seed of *Putranjiva roxburghii*. *Current Traditional Medicine*, *4*(4), 263–278.

Ezeh, K. J. and Ezeudemba, O. (2021). Hyperlipidemia: A Review of the Novel Methods for the Management of Lipids. *Cureus*, *13*(7), e16412.

Graf, B. L., Raskin, I., Cefalu, W. T. and Ribnicky, D. M. (2010). Plant-derived therapeutics for the treatment of metabolic syndrome. *Current opinion in investigational drugs (London, England: 2000)*, *11*(10), 1107–1115.

Grover, J. K., Yadav, S. and Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. *Journal of ethnopharmacology*, *81*(1), 81–100.

Hasimun, P., Sulaeman, A., Mulyani, Y., Islami, W. N., Apriany Timika Lubis, F. (2019). Antihyperlipidemic Activity and HMG CoA Reductase Inhibition of Ethanolic Extract of *Zingiber cassumunar* Roxb in Fructose-Induced Hyperlipidemic Wistar Rats. *Journal of Pharmaceutical Sciences and Research, 11*(5), 1897-1901.

HMGCR 3-hydroxy-3-methylglutaryl-CoA reductase [Homo sapiens (human)] - Gene - NCBI. (2018). HMGCR 3-hydroxy-3-methylglutaryl-CoA Reductase [Homo Sapiens (Human)] - Gene - NCBI.

Islam, B., Sharma, C., Adem, A., Aburawi, E. and Ojha, S. (2015). Insight into the mechanism of polyphenols on the activity of HMGR by molecular docking. *Drug design, development and therapy*, *9*, 4943–4951.

Islam, S., Rahman, S., Haque, T., Sumon, A. H., Ahmed, A. M. and Ali, N. (2020). Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. *Endocrinology Diabetes & Metabolism*, *3*(2), e00116.

Karam, I., Ma, N., Yang, Y. J. and Li, J. Y. (2018). Induce Hyperlipidemia in Rats Using High Fat Diet Investigating Blood Lipid and Histopathology. *Journal of Hematology and Blood Disorders*, *4*(1), 104

King, A. J. (2012). The use of animal models in diabetes research. *British Journal of Pharmacology*, *166*(3), 877–894.

Lefahal, M., Zaabat, N., Ayad, R., Makhloufi, E., Djarri, L., Benahmed, M., Laouer, H., Nieto, G. and Akkal, S. (2018). In Vitro Assessment of Total Phenolic and Flavonoid Contents, Antioxidant and Photoprotective Activities of Crude Methanolic Extract of Aerial Parts of *Capnophyllum peregrinum* (L.) Lange (Apiaceae) Growing in Algeria. *Medicines*, *5*(2), 26.

Lenzen, S. (2007). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*(2), 216-226.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H. and Chen, S. (2016). An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules*, *21*(10), 1374.

Lowry, O., Rosebrough, N., Farr, A. L. and Randall, R. (1951, November). Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry*, *193*(1), 265–275.

M.K. Unnikrishnan, Veeresh Veerapur, Yogendra Nayak, Piya Paul Mudgal, Geetha Mathew, Chapter 13 - Antidiabetic, Antihyperlipidemic and Antioxidant Effects of the Flavonoids, Editor(s): Ronald Ross Watson, Victor R. Preedy, Sherma Zibadi, Polyphenols in Human Health and Disease, Academic Press, 2014, Pages 143-161.

Mandal, A., Bhattarai, B., Kafle, P., Khalid, M., Jonnadula, S. K., Lamicchane, J., Kanth, R. and Gayam, V. (2018). Elevated Liver Enzymes in Patients with Type 2 Diabetes Mellitus and Non-alcoholic Fatty Liver Disease. *Cureus*.

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S. and Devasagayam, T. P. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of clinical biochemistry and nutrition*, *40*(3), 163–173.

Mohiuddin, G. S., Palaian, S., Shankar, P. R., Sam, K. G. and Kumar, M. (2019). Uncommon side effects of commonly used anti-diabetics: Time to monitor them. *International Journal of Pharmaceutical Science and Research*, *10*(9), 4145-4148.

Noeman, S. A., Hamooda, H. E. and Baalash, A. A. (2011). Biochemical Study of Oxidative Stress Markers in the Liver, Kidney and Heart of High Fat Diet Induced Obesity in Rats. *Diabetology & Metabolic Syndrome*, *3*(1).

Noordam, R., Vermond, D., Drenth, H., Wijman, C. A., Akintola, A. A., van der Kroef, S., Jansen, S. W. M.,

Huurman, N. C., Schutte, B. A. M., Beekman, M., Slagboom, P. E., Mooijaart, S. P. and van Heemst, D. (2017). High Liver Enzyme Concentrations are Associated with Higher Glycemia, but not with Glycemic Variability, in Individuals without Diabetes Mellitus. *Frontiers in Endocrinology*, *8*.

Ononamadu, C. J., Alhassan, A. J., Imam, A. A., Ibrahim, A., Ihegboro, G. O., Owolarafe, A. T. and Sule, M. S. (2019). In vitro and in vivo anti-diabetic and anti-oxidant activities of methanolic leaf extracts of *Ocimum canum*. *Caspian journal of internal medicine*, *10*(2), 162–175.

Parhofer, K. G. (2015). Interaction between Glucose and Lipid Metabolism: More than Diabetic Dyslipidemia. *Diabetes & Metabolism Journal*, *39*(5), 353.

Paun, G., Neagu, E., Albu, C., Savin, S. and Radu, G. L. (2020). In Vitro Evaluation of Antidiabetic and Anti-Inflammatory Activities of Polyphenolic-Rich Extracts from Anchusa officinalis and Melilotus officinalis. *ACS Omega*, *5*(22), 13014–13022.

Praparatana, R., Maliyam, P., Barrows, L. R. and Puttarak, P. (2022). Flavonoids and Phenols, the Potential Anti-Diabetic Compounds from *Bauhinia strychnifolia* Craib. Stem. *Molecules*, *27*(8), 2393.

Ravindra Babu (2012). Hypoglycemic Activity of Methanolic Extract of *Talinum triagulare* Leaves in Normal and Streptozotocin Induced Diabetic Rats. *Journal of Applied Pharmaceutical Science*.

Schlede, E., Genschow, E., Spielmann, H., Stropp, G. and Kayser, D. (2005). Oral acute toxic class method: A successful alternative to the oral LD50 test. *Regulatory Toxicology and Pharmacology*, *42*(1), 15–23.

Sekhon-Loodu, S. and Rupasinghe, H. P. V. (2019). Evaluation of Antioxidant, Antidiabetic and Antiobesity Potential of Selected Traditional Medicinal Plants. *Frontiers in Nutrition*, *6*.

Tundis, R., Loizzo, M. R. and Menichini, F. (2010). Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. *Mini Reviews in Medicinal Chemistry*,*10*(4), 315-331.

Wu, L. and Parhofer, K. G. (2014). Diabetic dyslipidemia. *Metabolism*, *63*(12), 1469–1479.

Yayinie, M., Atlabachew, M., Tesfaye, A., Hilluf, W., Reta, C. and Alemneh, T. (2022). Polyphenols, flavonoids, and antioxidant content of honey coupled with chemometric method: geographical origin classification from Amhara region, Ethiopia. *International Journal of Food Properties*, *25*(1), 76–92.

How to cite this article: Isha Talwar, Satish Kumar Sharma and Kaneez Fatima (2023). Evaluation of Anti-diabetic and Hypolipidemic Activity of *Drypetes roxburghii* Roots. *Biological Forum – An International Journal*, *15*(5): 1655-1669.