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(RESEARCH ARTICLE)

Brassica rapa juice decreases lipids and glucose levels with improved atherogenic index in rats

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Abstract

Stroke and coronary artery diseases are the primary causes of death among people with diabetes mellitus. Oxidative stress is one of the major causes of diabetic pathophysiology, while sulfur-containing compounds are vital in the treatment of oxidative-induced disorders. Turnip is rich in glucosinolates which are sulfur-containing compounds. Therefore current investigation focuses on the antidiabetic and hypolipidemic effects of the fresh juice of turnip. The juice of turnip was given through the oral route in three doses i.e. 4 ml/kg, 8 ml/kg, and 16 ml/kg according to body weight. Hyperglycemia and hyperlipidemia were induced by intraperitoneal injection of Alloxan. At the end of the experiment, the livers of the animals were randomly removed for histopathological examination. Serum lipid profile and blood glucose levels were tested along with atherogenic indices (Atherogenic index of plasma, Castelli's Risk Index, and Atherogenic coefficient) Microscopic examination of hepatic tissues revealed reduced intralobular inflammation. Whereas blood glucose levels, total cholesterol, triglycerides, and low-density lipoproteins were significantly decreased, high-density lipoproteins were significantly increased. Hence from the results of the present study, it may be concluded that turnip reduces blood glucose and lipid levels. It also improved atherogenic index showing cardio-protection and reduced liver inflammation exhibiting hepatic protection.

Keywords: *Brassica rapa*; Diabetes; Hypoglycemic; Hypolipidemic

1 Introduction

Diabetes can be described as a group of metabolic disorders due to inadequate insulin release or its resistance or both; eventually resulting in hyperglycemia[1] There are marked abnormalities in the metabolism of carbohydrate, lipid, and lipoprotein consequently leading to hyperlipidemia and atherosclerosis [2, 3] Many studies reveal the association of oxidative stress in the pathogenesis of diabetes by the alteration in enzymatic systems, lipid peroxidation, impaired Glutathione metabolism and decreased Vitamin C levels [4]. Uncontrolled diabetes mellitus may provoke cardiovascular diseases, stroke, many chronic diseases, and even death [5].

Diabetes is an emerging epidemic of the 21st century that has been affecting1 in every 11 people worldwide. Changes in lifestyle such as unhealthy diet and physical inactivity are deeply allied with the growing incidence of this disease [6].

WHO [7] estimated that diabetes would approximately double from 177 million in 2000 to 370 million in 2030; one more study predicted its probability to increase up to 439 million by the year 2030 [8]. Alloxan is popularly used to

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induce diabetes in animal models that allows observing variation in plasma glucose and lipid levels [9]. Alloxan after administration immediately reaches its target site destroying pancreatic β-cells [10].

Although the latest technology is being utilized to treat diabetes mellitus its severe consequences pose a direct threat to our next generations [11]. An ideal treatment for diabetes is a drug that not only controls glycemia but also prevents complications of diabetes. Currently available hypoglycemic agents are either too expensive or have undesirable side effects on chronic use [12]. Insulin injections are associated with the risk of hypoglycemia and impairment of hepatic and other body functions [13].

Before insulin, people from ancient times treated diabetes and its complications with indigenous remedies. Studies have proved the hypoglycemic and hypolipidemic potential of many herbs and plants [13]. Nearly 1200 plants are used globally to treat diabetes mellitus out of which many of them have been successfully tested in the laboratory to possess hypoglycemic effects [9].

Brassica rapa (BR) is one of the consumed root vegetables from the Brassicaceae family commonly known as a turnip. It is grown in temperate climates worldwide for its bulbous taproot. It contains valuable components which not only endorse health benefits but also provide healing properties [14]. It has a variety of organic compounds with biological activity such as flavonoids including isorhamnetin, kaempferol, and quercetin glycosides [14-18], glucosinolates, Phenylpropanoid derivatives, indole alkaloids, organic acids [19], and numerous minerals like copper, manganese, and calcium [14]. Turnip is medicinally used as an antiscorbutic, antiarthritic, and stimulant. It possesses several effects beneficial to human health including antioxidants [20], anti-inflammatory and anti-arthritic [17], anti-microbial and anti-fungal [21], hepatic protective [22, 23], and nephroprotective [16]. The current study was designed to investigate the antidiabetic and hypolipidemic potential of fresh juice of BR in alloxan-induced diabetic rats.

2 Material and methods

2.1 Ethical statement

The study was conducted after approval from the Board of Advance Studies and Research, University of Karachi. Reference no. 03294/Pharm was granted by the Board on May 02, 2017, to perform the study. This approval was followed by the permission of the Departmental Ethical Committee, Department of Pharmacology, for the use of animals as per the National Institutes of Health (NIH) guide for the care and usage of laboratory animals [24].

2.2 Animals

Adult male Wister rats with a mean bodyweight of 220±10 grams were purchased from Dow University of Health Sciences. The animals were acclimatized to laboratory conditions for a week before the start of the experiment. The environment was controlled to maintain a temperature of 23 ± 2° C, a humidity of 50-60%, and a 12h light/dark cycle. Animals were fed on standard food pellets and had free access to water, while husk and excreta were removed daily from the cages.

2.3 Brassica rapa juice

The roots of BR were purchased from the local market and identified by the center of plant conservation, the University of Karachi. The G.H.NO. 94831 was deposited in Herbarium and Botanic Garden, University of Karachi. The roots were washed then dried by blotting paper and squeezed after peeling to yield fresh juice. The filtered Juice was then given orally through a feeding tube in three doses 4 ml/kg, 8 ml/kg, and 16 ml/kg according to bodyweight [23].

2.4 Induction of Diabetes & Hyperlipidemia

Alloxan monohydrate (Sigma chemicals, USA) was employed for the induction of diabetes in rats. It was administered to overnight fasted rats as a single dose of 120mg/kg body weight through the intraperitoneal route [13] prepared freshly in normal saline. Rats after 72h were checked for glucose levels in plasma to confirm the induction of diabetes [25]. Rats showing plasma glucose levels greater than 250 mg/kg were thought to be diabetic and selected for the study. It was ascertained that alloxan-induced diabetic animals exhibit persistent hyperlipidemia; showing a considerable increase in total cholesterol (TC), triglycerides (TG), and Low-density lipoprotein (LDL) along with a decrease in high-density lipoprotein (HDL) [18].

2.5 Experimental Design

Rats were randomly distributed into 6 groups, each group having 10 rats. All animals were treated for 28 days as per the protocol given below: animals in the negative control group were simply given water for injection, animals in the positive control group, standard group, and treated groups were induced diabetes and hyperlipidemia and given water for injection, glibenclamide, and turnip juice respectively. Animals in both the negative and positive control groups received an equal volume of water for injection by mouth while the animals in the standard group received Glibenclamide in a dose of 10mg/kg orally and animals in the treated group received *Brassica rapa* juice in three doses 4 ml/kg, 8 ml/kg, and 16 ml/kg by oral route [3]. The whole experiment was carried out under NCCL guidelines [26]. Blood was withdrawn from the tail vein of rats on days 0, 7, 14, 21 & 28 [3] and glucose levels were measured by Accu-Chek glucometer. Percent reduction in blood glucose was assessed concerning the initial level [10]. The blood samples of all rats were drawn on the 28th day of the experiment through the cardio puncture to assess lipid profile. Serum was isolated using Humax 14 K and centrifuged at 2000 rpm for 10 min. Serum cholesterol, triglycerides, LDL, VLDL, and HDL were tested on Humalyzer 3000 (Human Germany), using standard testing kits supplied by Human, Germany. The Atherogenic indices were calculated as per the procedure adopted by Bhardwaj [27].

- Atherogenic Index of Plasma (AIP) = log (TG/HDL)
- Castelli's Risk Index (CRI-I) = TC/HDL
- Castelli's Risk Index (CRI-II) = LDL/HDL
- Atherogenic Coefficient (AC) = (TC– HDL)/HDL

Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess glucose tolerance. Overnight fasted rats were fed glucose (2 g/kg) orally and blood samples were taken from the tip of the tail at 0, 30, 60, and 120 min after the glucose loading dose [12].

2.6 Histopathological Examination

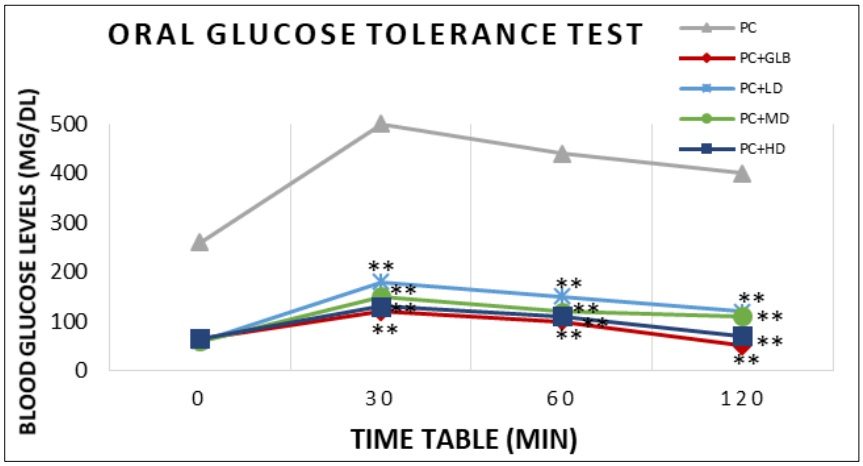
Livers were removed randomly one from each group, after completion of 28 days, and fatty tissues were removed for histopathological examination as described by Diab [28]. The histopathological damage was evaluated by a pathologist, unaware of the details of the experiment.

2.7 Statistical Analysis

Superior Performance Statistical Software (SPSS) version 20 was used to carry out data entry and analysis. Data were shown as mean ± SEM; the confidence interval was 95 %. For comparing the values with control, ANOVA was followed by post hoc. Value of p≤0.05 was supposed to be statistically significant and p≤0.005 highly significant.

3 Results

3.1 Oral Glucose Tolerance Test



Values represents mean ± S.E.M; n=10; *P ≤ 0.05 significant as compared to positive control, **P ≤ 0.005 highly significant as compared to positive control. PC= Positive control; GLB= Glibenclamide;
LD = BR 4ml/kg; MD=BR 8ml/kg; HD= BR 16ml/kg

Figure 1 Effect of BR on Glucose tolerance test

Figure 1 shows the effect of BR and glibenclamide on the oral glucose tolerance test. Fresh juice of BR (4ml/kg, 8ml/kg & 16ml/kg) and Glibenclamide (10mg/kg) produced a highly significant decrease (P≤0.005) in glucose levels in all glucose loaded rats. However, glucose tolerance was highly impaired in the positive control group.

3.2 Effect of *Brassica rapa* juice on Blood Glucose

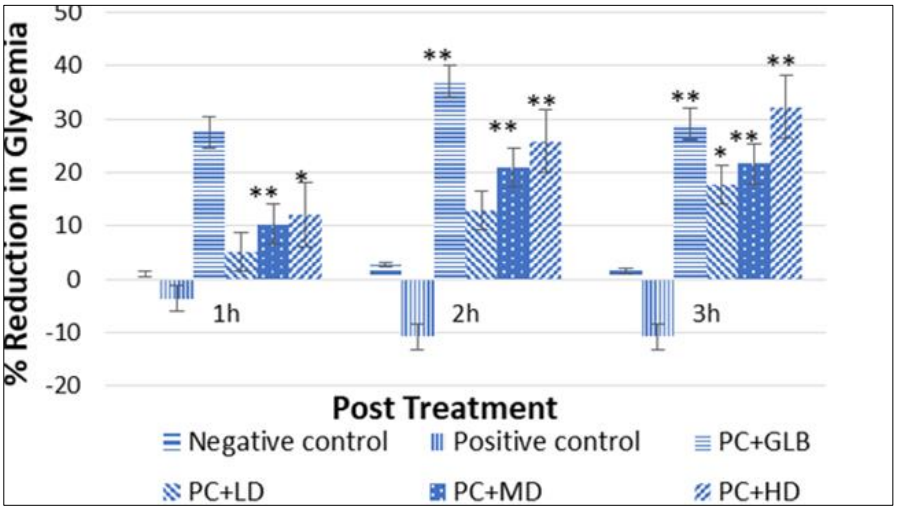
Table 1 shows the results of negative control, positive control, BR, and glibenclamide on blood glucose at different time intervals. A highly significant reduction in blood glucose levels was observed by glibenclamide and BR 8 ml/kg on days 7, 14, 21, and 28 as compared to positive control. While at 16ml/kg BR showed a significant decrease in BGL on day 14 and a highly significant decrease on days 21 and 28. However, at 4ml/kg BR showed a significant decrease in BGL only at day 28 as compared to positive control.

Table 1 Outcome of *Brassica rapa* on blood glucose levels of diabetic and control rats

Groups	Blood Glucose Levels (mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Negative Control	90.67±6.25	87.33±6.04	91±4.91	99.33±6.36	92.17±4.57
Positive Control	261.33±9.31	270.67±12.86	265.33±9.62	258.17±11.91	270.50±16.39
Glibenclamide	241±11.44	186.33±10.05**	126.67±7.03**	102.33±2.64**	87.50±2.04**
BR 4ml/kg	239±15.54	240±22.21	231.33±19.26	216.50±19.12	202.33±25.89*
BR 8ml/kg	218±5.56	193±5.26**	195±7.85**	152.50±6.67**	152.50±6.67**
BR 16ml/kg	246±11.55	217.50±10.70	205±10.16*	141.67±5.86**	111±3.60**

n=10, Values are mean ± S.E.M; *P ≤ 0.05 significant compared with positive control; **P ≤ 0.005 highly significant compared with positive control

Figure 2 shows the effect of BR on the blood glucose level of rats after a single oral dose. The percent reduction in blood glucose level was highly significant at 8 ml/kg and 16ml/kg, which was almost comparable to glibenclamide.



Values represents mean± S.E.M., n = 10; *p < 0.05 significant as compared to positive control; **p < 0.005 highly significant compared to positive control. PC= Positive control; GLB= Glibenclamide; LD= BR 4ml/kg; MD= BR 8ml/kg; HD= BR 16ml/kg

Figure 2 Effect of a single dose of BR on blood glucose level in rats concerning the initial (0 h)

3.3 Effect of BR Juice on Lipid Profile

Table 2 shows the effect of BR on lipid profile in hyperlipidemia-induced rats. There was a highly significant decrease in Total Cholesterol and LDL by *Brassica rapa* juice at 4ml/kg whereas a significant decrease was observed in TG and VLDL as compared to positive control.

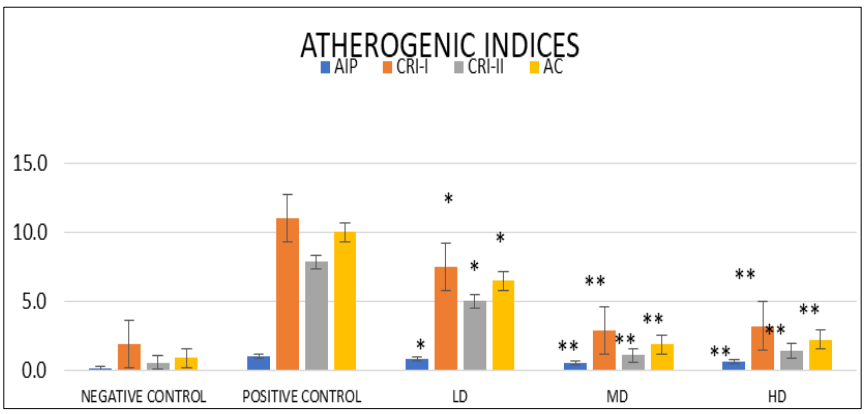
Brassica rapa at a dose of 8ml/kg showed a highly significant decrease in lipid profile, except HDL as compared to a positive control (Table 2). BR at 16ml/kg showed a highly significant decrease in TC and LDL levels, however, there was a significant decrease in TG & VLDL levels. While there was a highly significant increase in HDL as compared to positive control.

Table 2 Effect of *Brassica rapa* on lipid profile of hyperlipidemic rats

Groups	Parameters (mg/dl)				
	Total Cholesterol	Triglyceride	HDL	LDL	VLDL
Negative Control	55.91±2.65	46.28±2.34	29.326±1.08	17.33±1.78	9.25±0.46
Positive Control	140.05±2.66	136.25±2.27	12.71±0.93	100.08±2.43	27.25±0.45
BR 4ml/kg	94.75±3.54**	92.15±3.11*	12.66±1.11	63.65±3.36**	18.43±0.62*
BR 8ml/kg	55.86±4.196**	75.76±10.64**	19.266±0.91	21.44±4.46**	15.15±5.21**
BR 16ml/kg	78.9±5.73**	98.81±13.48*	24.33±3.21**	34.8±7.08**	19.76±6.6*

n=10, Values are mean ± S.E.M; *p ≤0.05 significant as compared to positive control; **p ≤0.005 highly significant as compared to the positive control

Figure 3 illustrates the effect of BR on atherogenic indices. There was a significant reduction in the atherogenic index of plasma by all three doses of BR as compared to positive control. While a highly significant decrease in Atherogenic Coefficient and Castelli's Risk Index was observed at 8ml/kg and 16ml/kg of BR as compared to positive control.



Values represent mean ± S.E.M; n=10. *P ≤ 0.05 significant; **P ≤ 0.005 highly significant as compared to positive control; Atherogenic Index of Plasma (AIP), Castelli's Risk Index (CRI-I), Castelli's Risk Index (CRI-II), Atherogenic Coefficient (AC), LD= BR 4ml/kg; MD= BR 8ml/kg; HD= BR 16ml/kg

Figure 3 Effect of *Brassica rapa* on Atherogenic indices

3.4 Histopathological Examination

Macroscopic examination of livers did not reveal any gross changes. However microscopic examination of hepatic tissues of some animals revealed mild to moderate changes. Animals in the negative control group did not exhibit any change in figure 4. Microscopic examination of rat hepatic tissue in the positive control group showed moderate intralobular inflammation figure 5. Hepatic tissues of animals that received 4ml/kg and 8ml/kg of BR exhibited minimal periportal inflammation figure 6, while there was no evidence of steatosis or periportal fibrosis in hepatic tissues of any animal.

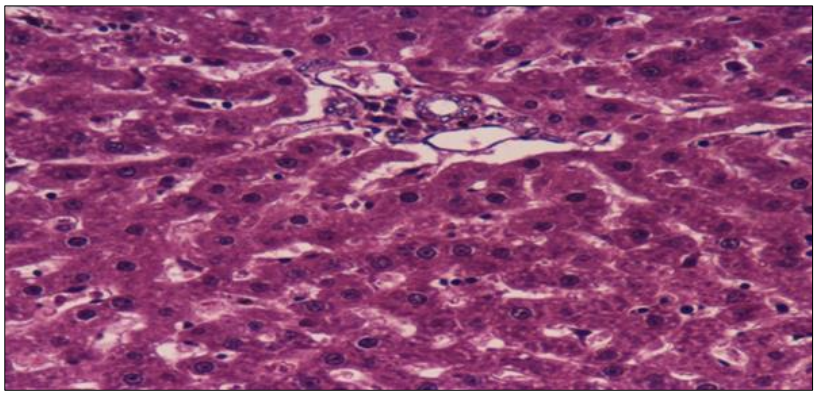


Figure 4 Hepatic tissue of rat showing intact portal tract with no microscopic changes

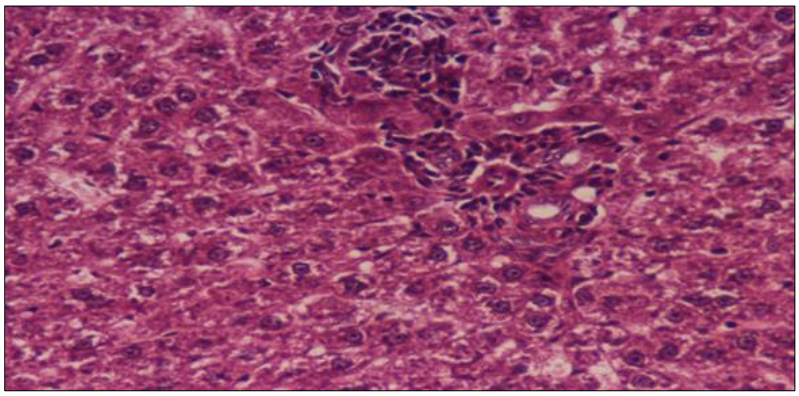


Figure 5 Hepatic tissue showing moderate per portal or intralobular inflammation

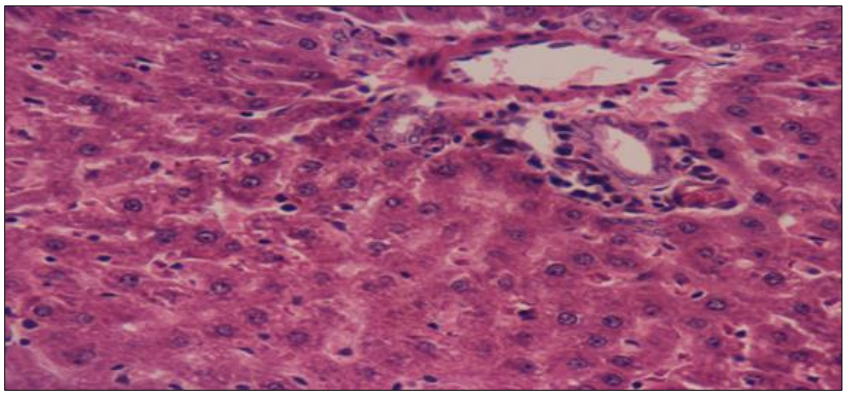


Figure 6 Hepatic tissue showing minimal periportal inflammation

4 Discussion

Diabetes mellitus is manifested by high blood glucose levels whether due to insufficient insulin release or insulin resistance; resulting in disturbed metabolism of both, glucose and lipids [9]. In diabetes, there is a surplus amount of hepatic glycogenolysis and gluconeogenesis along with low consumption of glucose by tissues [3, 9].

BR significantly reduced blood glucose in diabetic rats at all three doses following oral administration for 28 days. However, the results of 4ml/kg were highly significant and comparable to glibenclamide.

Several flavonoids and Sulfur compounds such as Glucosinolates are present abundantly in fresh juice of BR [15-19]. These compounds have shown hypoglycemic activity in several studies, such as sulfur derivatives have been found to reduce blood glucose by increasing insulin [13] Flavonoids like Quercetin & Kaempferol have been reported to exhibit hypoglycemic effect in alloxan-induced diabetic rats; while isorhamnetin inhibits aldose reductase which is becoming one of the therapeutic strategies proposed to prevent long-term diabetic complications [29]. Similarly, studies have shown that quercetin also improves insulin release in diabetic rats [11, 18, 30]; it also hinders glucose transporter GLUT-2 thereby declining glucose absorption from the intestine [11] Moreover, the possibility of BR to improve insulin secretion from pancreatic β-cells cannot be overlooked as a hypoglycemic mechanism. Since results of the present study are comparable to glibenclamide which produces hypoglycemia by improving the secretion of insulin from β-cells [31]. Thus, the hypoglycemic effect of BR can be attributed to its possible regeneration of surviving β -cells in the pancreas, potentiating insulin secretion [13], or decreasing intestinal absorption of glucose. This effect could be potentiated by flavonoids that help regenerate beta cells [3, 18].

Brassica rapa juice showed highly significant improvement in blood glucose levels within 2h of OGTT. The onset of action started after 30 min of the glucose load. These results were similar to those observed by Jung et al [30], who reported improved postprandial glucose by ethanol extract of Brassica rapa.

Oxidative stress is also one of the major complications of prolonged high blood glucose levels [32] and elevates the risk of atherosclerosis [33]. BR contains high amounts of vitamin C and other antioxidants such as phenolic compounds [34, 35] providing an additional benefit in combating diabetes.

Hepatic lesions resembling chronic liver diseases are one of the major pathological changes seen in DM. Alloxan is also reported to be involved in such lesions varying from the fatty degenerative changes of hepatocytes to steatohepatitis and liver fibrosis [36] Our drug BR in the moderate (8ml/kg) and high dose (16ml/kg) reduced periportal inflammation (interface hepatitis) exhibiting hepatoprotective effects.

Individuals with sustained hyperglycemia in diabetes are commonly shown to possess dyslipidemia [9] High levels of TGs and LDL and Low HDL levels are risk factors for cardiovascular diseases, particularly atherosclerosis [37]. Lack of insulin leads to insufficient activation of lipoprotein lipase (LL) responsible to hydrolyze triglycerides and high TGs levels [18]. Moreover, Insulin resistance results in uninhibited hormone-sensitive lipase (HSL) in adipocytes which eventually ends up in elevated lipolysis releasing more fatty acids; this augments the mobilization of FFA (free fatty acids) from peripheral depots to the liver [38, 39] rising serum lipids abnormally [32] In the liver, the fate of FFA is either esterification with glycerol synthesizing triglycerides or with cholesterol forming cholesteryl esters that accumulate to amplify hepatic lipid content [30, 32] Excess productions of TG also boosts the production of LDL cholesterol in the liver [9] Since inverse relation exists between total cholesterol and LDL with HDL, thereby HDL concentration goes down [32]. Therefore, measurement of lipid profile is essential in the management of diabetes to avoid cardiac complications [32] Drugs prescribed in diabetes such as oral sulfonylureas or insulin rectifies elevated triglycerides and reduced HDL but the abnormality in LPL remains uncorrected for months [40] evidence in literature reveals the beneficial effect of lipid-lowering therapy in diabetic individuals [41].

BR caused a highly significant reduction in triglycerides and LDL levels at 8ml/kg; whereas increased HDL highly significantly at 16ml/kg thus decreasing the chances of coronary heart diseases. These effects might be due to the presence of flavonoids present in BR [37] Flavonoids are known to improve hypercholesterolemia by effectively decreasing LDL, TC, and TGs [33, 42]. Studies reveal the inhibitory action of flavonoids and polyphenols on enzyme HMG CoA reductase thus reducing plasma cholesterol levels [18] Similarly, Quercetin also lowers cholesterol and LDL levels 33 Manganese present in BR have a role in improving insulin release and preventing lipids peroxidation reducing blood glucose level and cholesterol [43]. Moreover, the underlying hypolipidemic mechanism of BR might be due to increased secretion of insulin retarding lipid peroxidation, controlling lipolytic hormones, and reduced TG synthesis in the liver. Increased insulin also decelerates lipolysis in diabetic rats [13].

Studies have shown the role of atherogenic ratios as powerful indicators in predicting CAD and atherosclerosis. Lower values are indicative of the minimum tendency of cardiovascular diseases and protective action for the heart [37] When individual lipoproteins such as TG and HDL appear normal, AIP may be the diagnostic alternative [27] According to studies, TC/HDL ratio is more sensitive and specific parameter than TC, particularly when TG>300mg/dl in the identification of risk of CAD [27] Similarly, LDL/HDL ratio also plays a major role in identifying risk of CAD [43]. Our investigation revealed the potential of BR in significantly reducing AIP, CRI-I, CRI-II, and atherogenic coefficient at 8ml/kg and 16ml/kg.

5 Conclusion

The results of the present study suggest the promising role of *Brassica rapa* in diabetes with coexisting dyslipidemias because of its hypoglycemic and hypolipidemic effects. It also reversed the damage to hepatic tissues caused by alloxan showing hepatic protection. The current findings also suggest the cardio-protective role of *Brassica rapa* in diabetic subjects by decreasing the risk of dyslipidemias and atherosclerosis.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Authors Contribution

- AR: conceptualized the work and wrote the initial draft of the manuscript
- MB: carried out the practical work and performed the statistical analysis
- AAR: reviewed the manuscript
- RAK: corrected the initial draft and finalized the manuscript

Statement of ethical approval

Ethical approval was followed by the permission of the Departmental Ethical Committee, Department of Pharmacology, for the use of animals as per the National Institutes of Health (NIH) guide for the care and usage of laboratory animals.

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