Biochemistry and Biophysics Reports 22 (2020) 100755

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Dhanwantaram kashayam, an Ayurvedic polyherbal formulation, reduces oxidative radicals and reverts lipids profile towards normal in diabetic rats

Smitha Renganathan[a], Anand Srivastava[b], Radhakrishna Gopala Pillai[a,*]

[a] Department of Life Sciences, University of Calicut, Kerala, 673635, India
[b] Global Institute of Stem Cell Therapy and Research, 4660 La Jolla Village Drive, San Diego, CA, 92122, USA

ARTICLE INFO

Keywords:
Diabetes
Dhanwantaram kashayam
Antioxidant
Dyslipidemia
Antioxidant enzymes
Beta cells
Cholesterol
Triglycerides
Phospholipids

ABSTRACT

Background: Hyperglycemia and hyper oxidative stress are indicators of diabetes mellitus which is also accompanied with decreased levels of antioxidant enzymes. While oxidative stress is important in increasing insulin secretion and controlling blood sugar level at the same time excess oxidative stress leads to the destruction of beta cells of pancreas resulting in to low insulin production and hyperglycemia. A balance between the levels of oxidative radicals and insulin production is needed, but is not defined yet. Hyperglycemia also leads to hyperlipidemia which can contribute to various health conditions like cardiovascular diseases.
Objectives: This study was designed to study the oxidative stress and lipid levels in diabetic rats. This also was designed to elucidate the effect of Dhanwantaram Kashayam, an Ayurvedic polyphenolic derived from plants on lipid metabolism and oxidative radical scavenging in diabetic rats.
Methods: Rats were made diabetic by injecting streptozotocin. Different enzymes involved in oxidative radical scavenging and lipid profiles including triglycerides, total cholesterol, free fatty acids and phospholipids were estimated using standard methods reported elsewhere.
Results: Level of antioxidant enzymes were lower in diabetic rats compared to normal controls. Administration of Dhanwantaram Kashayam restored the enzyme activity as well as reduced levels of different lipids in diabetic rats.
Conclusions: Administration of Dhanwantaram Kashayam increased the activity levels of antioxidant enzymes and reduced the levels of total cholesterol, phospholipids and triglycerides. The results of this study point to the possibility of developing Dhanwantaram Kashayam as a dietary supplement which can alleviate the complications associated with diabetes or prevent them altogether.

1. Introduction

At global level incidences of metabolic disorders and related diseases are increasing at a rampant rate because of easy availability of rich food stuff and decreasing physical activity. Obesity and diabetes are considered as two main outcomes of metabolic disorders [1]. Disturbances in the metabolism of carbohydrate, lipid and protein are well reported in these metabolic disorders [2]. The β-cells of pancreas are highly metabolically active and depend on oxidative metabolism for generating ATP for their energy requirements [3]. Elevated glucose level stimulates the production of insulin [3] and at the same time the enhanced production ROS is an unavoidable process in mitochondrial metabolism during high glucose tion [4] Enzymes of antioxidant metabolism are expressed at very low level in pancreatic β-cells [5]. These two conditions combined together render the β-cells highly susceptible to damages caused by oxidative stress [3].

Oxidative stress plays an important role in tissue damages associated with diabetes [6]. An increase in reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion, and hydroxyl radicals have been reported in different cells exposed to high glucose [7,8]. Healthy cells have developed defensive mechanisms to manage the over production of ROS which include both enzymatic or non-enzymatic antioxidant systems. Enzymatic antioxidant defense mechanisms include enzymes such as superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione Reductase (GRd) etc [9,10]. SOD converts the highly reactive radicals O_2^- to less reactive molecules H_2O_2, which is then converted to harmless molecules like water and oxygen by CAT and GPx [11].

Dyslipidemia due to abnormal lipid metabolism is a prominent characteristic of diabetes [12]. This includes elevated levels of total

* Corresponding author.
E-mail address: pillai_radhakrishna@hotmail.com (R.G. Pillai).

https://doi.org/10.1016/j.bbrep.2020.100755
Received 16 December 2019; Received in revised form 16 March 2020; Accepted 19 March 2020

cholesterol and triglycerides which are major contributory risk factors leading to cardiovascular diseases [13]. To prevent complications in patients with diabetes these risk factors are needed to be strictly controlled.

Till now there is no effective treatment for life-long recovery from Diabetes Mellitus. Diabetic patients have an increased risk of developing a number of serious life-threatening health problems resulting in increased mortality [14]. The global prevalence of diabetes is increasing day by day. The number of peoples with diabetes has increased four times during the last three decades [15] and the number is feared to reach 587 million diabetic adults (18–99 years) by the year 2045, if no preventive measures are taken [16,17].

Traditional medications derived from plant sources are increasingly gaining importance due to their potential efficacy in the management of many chronic ailments without inciting many undesired side effects including diabetes [17,18]. Dhanwantaram Kashayam (DK), a polyherbal decoction described in the classical Ayurvedic text "Astanga Hridaya" [19], consists of 40 herbal ingredients which is used in Ayurveda as a growth stimulant for children and for nerve regeneration [20]. The strong antioxidant activities of DK and its ability to scavenge free radicals in the body have already been reported [21]. Research during the last two decades has indicated the influence of hyperglycemia and hypoxia in the sensing and production of insulin and the health of pancreatic beta cells. The extent of antioxidant status and its exact effect on the beta cell function is not fully understood. The main objective of this study was to explore the effects of DK on ROS load in diabetic rats and their response in terms of lipid profile.

2. Materials and methods

2.1. Chemicals

All chemicals used for this study were of analytical grade and purchased from Sisco Research Laboratories (Mumbai, India).

2.2. Experimental animals

Rats of Wistar strain (200–220 g) maintained in the departmental animal house (Room temperature 25 ± 5^{0}C with 12 h light and dark cycles) were used for the study. Male and female rats were housed separately in hygienic polypropylene cages, provided with rodent food (VRK Nutritional Solutions, Maharashtra, India) and water *ad libitum*. All procedures involving animals were carried out in accordance with the guidelines on the care of laboratory animals and their use for scientific purpose. Single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (Sisco Research Laboratories, Mumbai, India) at a dose of 40 mg/kg of body weight, prepared in 0.1 molar citrate buffer, pH 4.5 [22] to rats deprived of food overnight made them diabetic. Rats were immediately supplied with 5% of glucose in drinking water for the first 24 h to safe guard against initial hypoglycemia. Blood glucose was measured by using "Dr. Morepen Gluco One Blood glucose monitoring system" and BG-03 model glucose strips (Morepen Laboratories Limited, Delhi). The animals with blood glucose level > 300 mg/dl on the third day were classified as diabetic. One subgroup of animals of each group did not receive any DK treatment and served as corresponding control. With three dose levels of DK to be used in two different groups, non-diabetic and diabetic, made altogether 8 groups of animals. DK, freshly diluted with sterile water (1:3), was administered orally for 21 days at a dosage of 1 ml (low dose or Dose A), 1.5 ml (intermediate or Dose B) and 2 ml/kg of body weight (high or Dose C) respectively twice a day before allowing any food for at least 4 h. Each group consisted of 6 rats.

2.3. Animals groups & experimental design

Animals of each sex were divided into eight groups namely;

Experimental group I: Normal Control (NC)
Experimental group II: Diabetic-Control (DC)
Experimental group III: Diabetic + DK Dose A (DDKA)
Experimental group IV: Diabetic + DK Dose B (DDKB)
Experimental group V: Diabetic + DK Dose C (DDKC)
Experimental group VI: Control + DK-Dose A (CDKA)
Experimental group VII: Control + DK- Dose B (CDKB)
Experimental group VIII: Control + DK- Dose C (CDKC)

On 21st day they were deprived of food overnight and sacrificed on the following morning. Blood samples were individually collected in centrifuge tubes. Tissues were collected in ice cold containers.

2.4. Analysis of levels of antioxidant enzymes and lipid profile

All estimations were done separately for groups of male and female rats. Analyses of levels of enzymes responsible for carrying out antioxidative activities and Lipid profiles were carried out by using standard protocols;

Super Oxide Dismutase (SOD) [23], Catalase (CAT) [24], Glutathione Peroxidase (GPx) [25], Glutathione reductase (GRd) [26], Total cholesterol (TC) [27], Free Fatty Acids (FFAs) [28], Phospholipids (PLs) [29] and Triglycerides (TGs) [30].

2.5. Statistical analysis

Data are represented as Mean ± SD (Standard Deviation) of the values recorded in 6 animals. The results were analyzed by using SPSS -version 21 software for windows. One - way ANOVA (analysis of variance) followed by Tukey Post hoc multiple comparison test was used to decide statistically significant changes between the groups. Values with $P < 0.05$ were considered as statically significant.

3. Results

No significant differences in the results were observed between male and female rat groups, so the results are shown as one set.

3.1. Catalase

CAT is an enzyme which is responsible for conversion of hydrogen peroxide into oxygen and water. We studied level of CAT in liver, kidney, heart and serum of normal and diabetic rats. Activities of CAT were ranging in a tissue specific manner (Fig. 1). Its levels normal rats were 73 ± 2, 25 ± 1.8, 57 ± 3.5 and 113 ± 11 U/mg protein in liver, kidney, heart and serum respectively. Not surprisingly, the activity of CAT decreased in diabetic rats when compared to that in normal control rats which was in a good agreement with previous reports [5]. The levels of CAT dropped more than 75% in serum. To study the effectiveness of DK different doses of diluted decoction were given to rats by gavage. Administration of DK led to a significant increase ($p < 0.001$ and $P < 0.05$) in the activity of CAT in all tissues and serum of both diabetic and normal rats. Though there was some correlation between the dose of the DK and increase in CAT activity, but the rate of increase varied in different tissues and serum of normal rats and diabetic rats. There was no significant difference in the CAT levels in liver and serum of diabetic rats receiving intermediate dose (DDKB) and non-diabetic rats receiving highest dose (CDKC) rats in comparison with normal rats. CAT levels in the kidneys of group 3 and 4 were very close to diabetic rats. Administration of DK increased catalase activity in all the tissues. In diabetic rats the highest percentage increase was observed in highest dose fed ones.

3.2. SOD

SOD is an enzyme that alternately catalyzes the dismutation of the

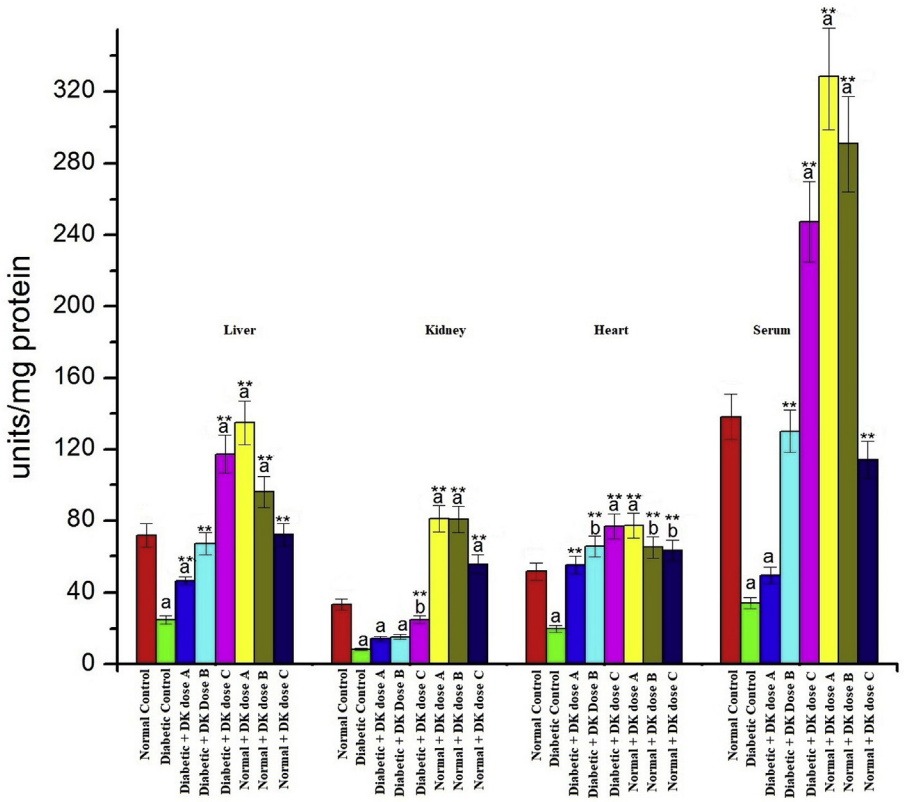

Fig. 1. Showing the catalase levels in the different tissues of rats subjected to different treatments. Values are expressed as mean ± SD of six rats. 'a' indicates values are significantly different from Normal Control rats with $p < 0.001$. 'b' indicates values are significantly different from Normal Control rats with $p < 0.05$. '**' indicates values are significantly different from Diabetic Control rats with $p < 0.001$. '*' indicates values are significantly different from Diabetic Control rats with $p < 0.05$. One unit of enzyme is defined as the μM of hydrogen peroxide consumed per minute.

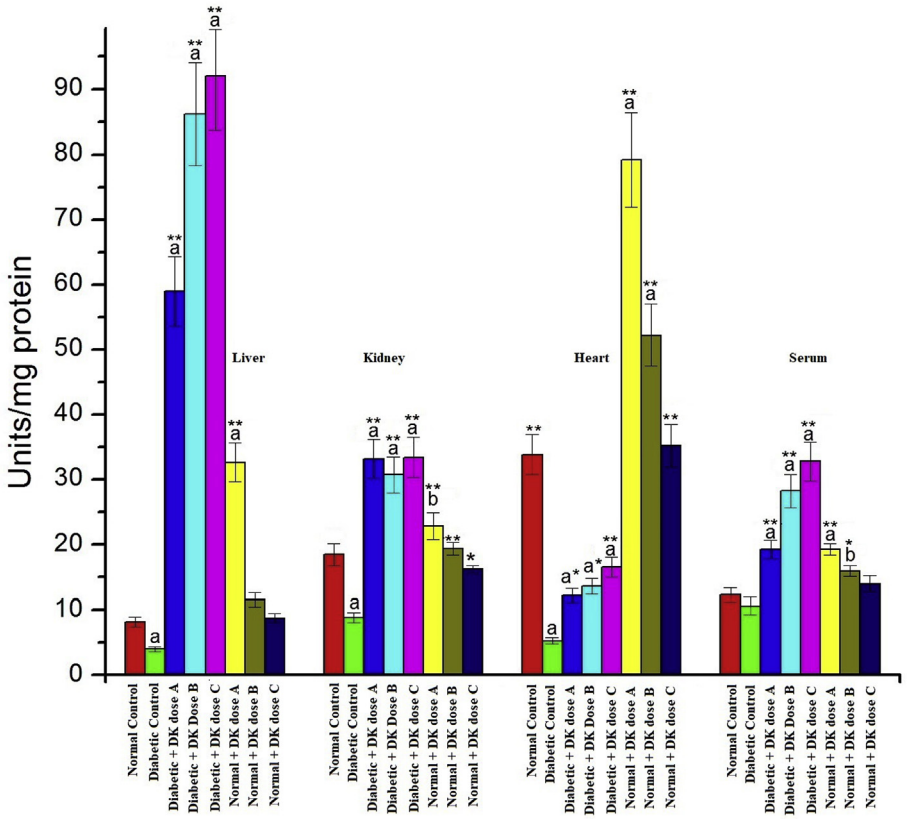

Fig. 2. Showing the SOD levels in the different tissues of rats subjected to different treatments. Values are expressed as mean ± SD of six rats. 'a' indicates values are significantly different from Normal Control rats with $p < 0.001$. 'b' indicates values are significantly different from Normal Control rats with $p < 0.05$. '**' indicates values are significantly different from Diabetic Control rats with $p < 0.001$. '*' indicates values are significantly different from Diabetic Control rats with $p < 0.05$. One unit is the enzyme required to inhibit the chromogen production by 50% in one minute.

superoxide (O_2^-) radicals into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). There were tissue specific variations in the levels of SOD as seen in case of catalase. In normal rats SOD activities were 8.2 ± 0.74, 18.4 ± 1.68, 33.82 ± 3.08 and 12.3 ± 1.12U/mg protein in liver, kidney, heart and serum respectively (Fig. 2). Activity of SOD decreased drastically in diabetic rats

which were 4.01 ± 0.037, 8.81 ± 0.8, 5.25 ± 0.47 and 10.59 ± 1.332U/mg protein in liver, kidney, Heart and serum respectively. Administration of DK increased SOD activity in all the rats. In diabetic rats increase in SOD reflected the increasing dose in all tissues except in kidney. In kidney all the DK doses caused a similar increase and the increase caused by different doses was significantly different from diabetic control and normal control ($p < 0.001$ and $P < 0.05$). 5–10 times increase in SOD activity was observed in the liver of diabetic rats fed DK with the highest dose producing the highest increase. In normal rats low dose of DK (dose-A) produced the highest increase in SOD activity. Higher doses also caused an increase in SOD activity, but the increase was not as high as in dose A fed rats. The change in SOD activity in the liver of group DC, CDKB and CDKC are not significant compared to NC. In the case of heart there was no significant difference between normal and group CDKC rats. DK administration increased the SOD level. No significant difference in SOD activity in the serum of groups DC and CDKC compared to NC. In normal rats the lowest dose of DK caused the highest increase in SOD activity in all the tissues studied. The rate of increase in SOD activity decreased with increasing dose of DK.

3.3. Glutathione peroxidase

Glutathione peroxidase reduces oxygen free radical containing molecules in corresponding alcohols. It also converts hydrogen peroxide into water. The levels of its activities varied due to DK administration in different tissues but those were not as pronounced as that in cases of CAT and SOD. In normal rats, its activities were recorded to be 9.52 ± 0.86, 10.4 ± 0.52, 3.49 ± 0.319 and 7.01 ± 0.64 U/mg protein in liver, kidney, heart and serum respectively (Table 1). Its activities decreased drastically ($p < 0.001$) in diabetic rats as compared to normal controls except in serum. Its activity dropped to 2.8%, 10% and 3.8% in liver, kidney and heart but to about 75% in serum. Here also an increase in the enzyme activity in diabetic rats because of administration of DK and the increase in glutathione peroxidase was more in non-diabetic rats than in diabetic animals. In normal rats the DK administration increased the enzyme activity most effectively by the lowest dose used in the study (dose A) ($P < 0.001$). In normal rats, higher doses of DK produced varying effects in different tissues. In liver and kidney intermediate dose (dose B) also resulted a significant increase, but, interestingly, the highest dose used (dose C) did not produce any noticeable change in activity of this enzyme. In heart of normal rats all the doses produced significant increase ($p < 0.001$), but the pattern of increase was similar to that observed in liver and kidney ie., dose A producing highest effect and the level of increase was lesser in dose B and least in dose C fed rats. In serum also DK administration increased the enzyme activity, but to a lower level compared to that in other tissues studied. No significant difference was observed in serum of DK fed normal rats.

3.4. Glutathione Reductase

There was a tissue specific variation in activity of GRd also. Enzyme activities were recorded to be 7.30 ± 0.39, 10.14 ± 0.92, 8.7 ± 0.36, and 2.55 ± 0.106 U/mg protein in liver, kidney, heart and serum respectively (Fig. 3). GRd activity significantly ($p < 0.001$) declined in all tissues except in kidneys of diabetic rats when compared to normal controls. However the decrease was not as pronounced as seen in case of other enzymes. This decrease in GRd activity was also reversed by DK administration. Even the lowest dose used produced a significant increase ($p < 0.001$). The increase in GRd activity with DK administration reflected some dose and tissue dependence. In normal rats DK administration increased the GRd activity, but the pattern of increase was different from that observed in DK fed diabetic rats where the increase in activity was proportional to the amount of medication gavaged in rats. In DK fed normal rats, the lowest dose produced the highest increase in enzyme activity. In heart all the doses produced almost similar increase.

3.5. Lipid profiles

A significant increase in fatty acids, lipids and cholesterol in tissues and serum during diabetic conditions is a very well known undesired outcome. Lipid profiles such as TC, FFA, TG and PL concentrations were analyzed in diabetic rats and compared with those in normal control rats. Also, effect of administration of DK on lipid profile values in normal control and diabetic rats was studied.

3.5.1. Total cholesterol (TC)

Level of cholesterol in tissues was in range of 3–6 mg/g of wet weight and 40–53.6 mg/dl in serum. In diabetic rats the level increased by 19.2, 79.7, 37.7 and 23.2% in liver, kidney, heart and serum respectively (Table 2). A significant increase ($P < 0.001$ and $P < 0.05$) in TC was observed in all the tissues analyzed except in serum of diabetic rats compared to normal. Administration of DK reduced this increase in a dose dependent manner. High dose of DK decreased the TC level below normal in all the tissues analyzed except in serum where the decrease was not significant. Similar effects were observed in DK fed normal rats. In normal rats, the DK administration reduced the TC level in all the tissues except in serum where the dose A resulted and increase in TC.

3.5.2. Free fatty acids

Levels of FFAs displayed tissue related variations. Its levels were 5.742 ± 0.228, 6.07 ± 0.241, 2.26 ± 0.09 mg/g tissue in liver, kidney and heart respectively (Table 3). In serum of normal rats, the FFA was 67.6 ± 2.68 mg/dl. Changes in FFA levels were in a similar pattern as seen in case of TC. Increases in the FFAs by 53.4, 29.48, 107.7 and 43.04% in liver, kidney, heart and serum respectively were seen in diabetic rats and the FFA level increase was statistically significant ($P < 0.001$). Administration of DK to diabetic rats reduced the FFA level in all tissues studied. The decrease was in a dose dependent manner in heart, kidney and serum. In liver FFA level was brought to normalcy by dose A, but higher doses did not make any further effect. Hence this result demonstrates a tissue-specific effectiveness of the preparation. The highest dose brought down the FFA level to normal or

Table 1
Glutathione Peroxidase (Units/mg protein).

SL.NO	GROUPS	LIVER	KIDNEY	HEART	SERUM
1	NORMAL CONTROL	9.52 ± 0.87 **	10.41 ± 0.53 **	3.5 ± 0.32**	7.02 ± 0.64
2	DIABETIC CONTROL	0.27 ± 0.03[a]	1.41 ± 0.07[a]	0.09 ± 0.01[a]	5.31 ± 0.49[a]**
3	DIABETIC + DOSE A	8.49 ± 0.78**	10.38 ± 0.52 **	0.40 ± 0.37[a]	5.41 ± 0.49[a]
4	DIABETIC + DOSE B	7.95 ± 0.72 **	9.92 ± 0.50 **	0.72 ± 0.07[b]	5.79 ± 0.53[b]
5	DIABETIC + DOSE C	13.85 ± 1.26[a]**	10.61 ± 0.54**	1.36 ± 0.13[b]	6.47 ± 0.59 *
6	NORMAL + DOSE A	21.75 ± 1.99[a]**	15.68 ± 0.79[a]**	26.54 ± 2.42[a]**	7.13 ± 0.65 **
7	NORMAL + DOSE B	15.63 ± 1.43[a]**	12.78 ± 0.65[a]**	19.39 ± 1.77[a]**	6.45 ± 0.59*
8	NORMAL + DOSE C	9.72 ± 0.89 **	10.87 ± 0.55 **	15.70 ± 1.43[a]**	6.36 ± 0.58 *

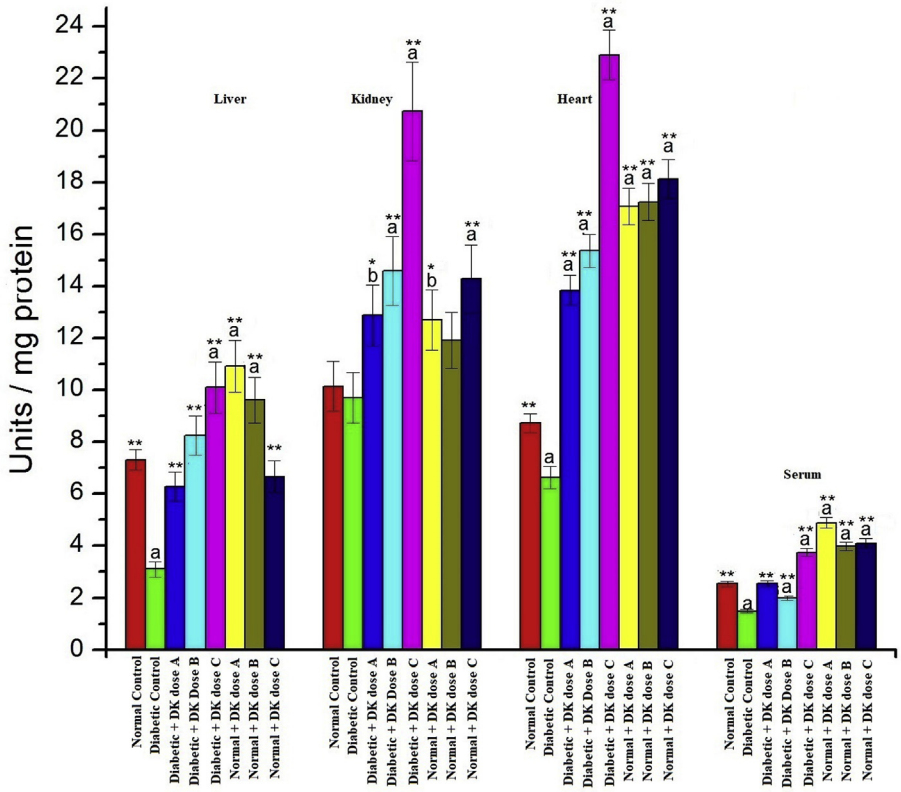

Fig. 3. Showing the levels of glutathione reductase in the different tissues of rats subjected to different treatments. Values are expressed as mean ± SD of six rats. 'a' indicates values are significantly different from Normal Control rats with $p < 0.001$. 'b' indicates values are significantly different from Normal Control rats with $p < 0.05$. '**' indicates values are significantly different from Diabetic Control rats with $p < 0.001$. '*' indicates values are significantly different from Diabetic Control rats with $p < 0.05$. One unit is the μM of NADPH oxidised per minute.

even below in non-diabetic rats. Administration of DK in normal rats did not affect the FFA level in liver and heart whereas in kidney and serum the FFA level decreased significantly ($P < 0.001$ and $P < 0.05$).

3.5.3. Phospholipids

PL level increased significantly ($p < 0.05$) in the liver, heart and serum of diabetic rats (Table 4), but no significant change was observed in kidney. DK administration significantly ($P < 0.001$ and $P < 0.05$) decreased PL levels in all tissues in both diabetic and non diabetic rats in a dose dependent manner. In liver and heart, dose B was able to bring the PL to a level below normal, but in serum a higher dose (dose C) was need to bring the PL level to normalcy. The decreases in diabetic rats due to the highest dose of DK were 27%, 34%, 25 and 22% in liver, kidney, heart and serum respectively. All doses of DK decreased PL far below normal levels in the different tissues except heart and serum. In heart and serum the dose A and B resulted a decrease in PL level, but remained above or same as normal levels.

3.5.4. TG

Levels of TG varied in different target tissues. Its levels were 2.78 ± 0.1, 3.32 ± 0.12 and 2.23 ± 0.79 mg/g of wet weight in liver, kidney and heart and 50.356 ± 1.78 mg/dl in serum (Table 5). A significant increase in TG levels was observed in all the tissues of the diabetic rats. The increases were 112.8%, 87.4%, 65.02% and 32% in liver, kidney, heart and serum respectively. TG levels in the liver remained normal in DK administered normal rats. A significant ($P < 0.001$ and $P < 0.05$) decrease in the TG level in the liver, kidney, heart and serum was observed in DK fed diabetic rats compared to diabetic control rats. In kidney and heart of diabetic rats the dose C was sufficient to bring the TG level to normal. TG levels in the serum of all rats except DDKA showed significant difference ($P < 0.001$ and $P < 0.05$) from NC. In heart and serum, the dose C brought the TG level to about 50% of normal value.

4. Discussion

Around the globe incidences of diabetes is increasing at a rampant rate which is causing a big financial and social dent in infrastructure of the society. Almost all organs of a body are adversely affected by diabetes which leads to a number of health related problems. Though a number of pharmacological agents are in use to minimize the ill-effects brought about by diabetes, they have some degree of undesired side effects. In order to find an additional therapeutic help from conventional Indian Medical literature, we chose to evaluate an Ayurvedic preparation in diabetic rats.

Table 2
Total cholesterol.

SL.NO	GROUPS	LIVER	KIDNEY	HEART	SERUM
1	NORMAL CONTROL	5.21 ± 0.18 **	5.94 ± 0.21 **	6.1 ± 0.22 **	43.61 ± 1.55 **
2	DIABETIC CONTROL	6.23 ± 0.22[a]	9.71 ± 0.34[a]	8.36 ± 0.3[a]	53.77 ± 1.91[a]
3	DIABETIC + DOSE A	5.17 ± 0.18**	6.74 ± 0.24[a]**	7.02 ± 0.25[a]**	52.16 ± 1.85[a]
4	DIABETIC + DOSE B	4.6 ± 0.16[a]**	6.03 ± 0.21 **	5.95 ± 0.21 **	50.74 ± 1.8[a]
5	DIABETIC + DOSE C	4.46 ± 0.16[a]**	5.79 ± 0.21 **	6.23 ± 0.22 **	51.56 ± 1.83[a]
6	NORMAL + DOSE A	4.85 ± 0.172[b]**	4.37 ± 0.16[a]**	6.18 ± 0.22 **	50.38 ± 1.79[a]*
7	NORMAL + DOSE B	3.88 ± 0.14[a]**	5.61 ± 0.19 **	5.63 ± 0.2[b]**	41.83 ± 1.48 **
8	NORMAL + DOSE C	3.92 ± 0.14[a]**	5.33 ± 0.19[a]**	4.98 ± 0.18**	40.04 ± 0.42[b]**

Table 3
Free Fatty Acids (mg/g tissue/dL serum).

SL.NO	GROUPS	LIVER	KIDNEY	HEART	SERUM
1	NORMAL CONTROL	5.74 ± 0.23 **	6.07 ± 0.24 **	2.26 ± 0.09 **	67.61 ± 2.68**
2	DIABETIC CONTROL	8.83 ± 0.35[a]	7.86 ± 0.31[a]	4.69 ± 0.19[a]	96.66 ± 3.83[a]
3	DIABETIC + DOSE A	5.10 ± 0.20[a]**	5.64 ± 0.22[b]**	2.55 ± 0.10[a]**	85.38 ± 3.38[a]**
4	DIABETIC + DOSE B	6.11 ± 0.24**	4.03 ± 0.16[a]**	2.53 ± 0.10[b]**	78.13 ± 3.1[a]**
5	DIABETIC + DOSE C	5.33 ± 0.21**	3.52.14[a]**	2.29 ± 0.09 **	67.98 ± 2.7 **
6	NORMAL + DOSE A	5.24 ± 0.21[b]**	2.82 ± 0.11[a]**	2.54 ± 0.10[b]**	60.33 ± 2.39[b]**
7	NORMAL + DOSE B	5.28 ± 0.21[b]**	2.90 ± 0.12[a]**	2.43 ± 0.1 **	58.76 ± 2.33[a]**
8	NORMAL + DOSE C	5.13 ± 0.20[a]**	2.56 ± 0.10[a]**	2.19 ± 0.09 **	59.42 ± 2.36[a]**

It is well established fact that increased oxidative stress, also observed in diabetes suffering individuals, induces dysfunction of the β-cells and contributes to the progression of diabetes [31]. Decreased level of antioxidant enzymes observed in the diabetic rats recorded in present study is in a good agreement with the previous reports [5]. The main functions of the free radical scavenging enzymes such as SOD, CAT, GPx, GRd are to protect the biological molecules and there by the whole system from free radical-induced damages [32,33]. SOD converts the most detrimental superoxide anions in to less toxic hydrogen peroxide and oxygen while the heam –containing ubiquitous enzyme CAT detoxifies H_2O_2 by converting it into water and oxygen [34]. The antioxidant enzyme GPx also scavenges H_2O_2. In addition to that, GPx is also involved in decreasing the levels of lipid-peroxides and organic hydroperoxides [35]. Another important antioxidant enzyme, GRd, helps in recycling of oxidised glutathione back into glutathione [36].

In the present study, we investigated the effect of DK in rat model of diabetes. In this study we mainly focused on parameters like antioxidant enzymes and lipid profiles. Antioxidant enzymes like CAT, SOD, GPx, GRd were decreased in diabetic rats, but DK administration reversed this. Antioxidants help biological system to repair damages caused by the free radicals [37]. Deleterious effects of free radicals depend on their levels which is counteracted by the ability of the cells to detoxify these radicals by employing the enzymes involved in free radical transmutation [38]. DK administration increased the levels of the antioxidant enzymes. This in turn might have removed the toxic compounds from the body which explains the low levels of these compounds observed in DK administered rats.

Animals that are made diabetic by injecting STZ are commonly used as typical animal disease model for studying diabetes [39]. Enhanced production of ROS by the injected STZ induces cytotoxicity in pancreatic β-cells leading to reduced insulin production [40]. Antioxidants and free radical scavengers help protect pancreatic Islets from the action of STZ and also help in the regeneration of the pancreatic β-cells [41]. Active ingredients of most herbal ingredients of DK have strong antioxidant activities [21]. Increase in antioxidant parameters in DK fed diabetic and normal rats may be due to two reasons. DK may act as a powerful antioxidant in biological system which may help in the removal of the free radicals in DK fed rats. So the turnover rate of natural antioxidants in the body was decreased. Another way could be by direct stimulation of the activity of CAT, SOD, GPx and GRd by DK which, in turn, could have removed the free radicals.

Lipid profiles of various molecules like TC, FFA, TG and PL demonstrated that these were increased in diabetic rats. It is a well established fact also observed in human beings. The increased lipid levels have a inhibitory effect on insulin production [42]. DK administration decreased lipid profiles in diabetic rats and also had reduced blood glucose level. This decrease in lipid levels might have removed the inhibition on insulin production which, in turn, resulted in reduced glucose levels. Dyslipidaemia, a common phenomenon observed in patients having insulin deficiency or insulin resistance, plays a major role in developing cardiovascular diseases (CVD) in diabetic patients [43,44] CVD has been found to be the most prevalent causative factor in diabetes related mortality [45]. The observed decrease in lipid levels due to DK administration indicates its ability to reduce CVD in diabetic patients and hence the related mortality.

In a later study the experiments were repeated in female rats of the same strain. The results are not reported as there were no significant differences in between the sexes.

DK is rich in phytochemicals such as polyphenols, tannins, flavanoids etc [21]. Polyphenols have anti-lipidemic activity [46,47]. Tannins decrease dietary absorption of cholesterol [48] The presence of large amount of phytochemicals [48] with excellent antioxidant activity may be the reason for the antihyperlipidemic activity of DK in diabetic and normal rats.

It is also a point to keep in one's mind that it is almost impossible to determine the amount and nature of active ingredients of herbal preparations in general which may also in case of DK. Also, the time of harvesting those herbs and method of preparation of decoction may contribute to batch to batch variations.

5. Conclusion

The present study has shown a decrease in the activity of antioxidant enzymes in diabetic rats whereas lipid profile demonstrated enhanced levels of cholesterol and lipids. Administering DK to diabetic rats resulted in a reversal of the situation. From these results, it can be concluded that DK, a polyherbal formulation used in Ayurvedic practice for centuries, exerts significant antioxidant and anti hyperlipidemic activity. This herbal product could be developed as a promising natural and safe remedy or as a preventive agent in diabetes mellitus.

Table 4
Phospholipids (mg/g tissue/dL serum).

SL.NO	GROUPS	LIVER	KIDNEY	HEART	SERUM
1	NORMAL CONTROL	9.02 ± 0.31 **	10.11 ± 0.34 *	13.1 ± 0.47 **	95.27 ± 3.22 **
2	DIABETIC CONTROL	11.44 ± 0.39[a]	10.75 ± 0.36[b]	15.66 ± 0.53[a]	109.15 ± 3.69[a]
3	DIABETIC + DOSE A	10.35 ± 0.35[a]**	8.66 ± 0.29[a]**	15.29 ± 0.52[a]	107.51 ± 3.63[a]
4	DIABETIC + DOSE B	8.33 ± 0.28[b]**	8.46 ± 0.29[a]**	13.19 ± 0.45 **	104.15 ± 3.52[a]
5	DIABETIC + DOSE C	7.48 ± 0.25[a]**	8.45 ± 0.29[a]**	12.64 ± 0.45[a]**	97.22 ± 3.29 **
6	NORMAL + DOSE A	6.75 ± 0.23[a]**	7.84 ± 0.267[a]**	12.18 ± 0.41[a]**	77.72 ± 2.63[a]**
7	NORMAL + DOSE B	6.53 ± 0.3[a]**	7.58 ± 0.26[a]**	11.1 ± 0.38[a]**	78.17 ± 2.64[a]**
8	NORMAL + DOSE C	6.63 ± 0.22[a]**	6.52 ± 0.22[a]**	10.52 ± 0.37[a]**	74.51 ± 2.52[a]**

Table 5
TG (mg/g tissue/dL serum).

SL.NO	GROUPS	LIVER	KIDNEY	HEART	SERUM
1	NORMAL CONTROL	2.78 ± 0.1 **	3.32 ± 0.12 **	2.23 ± 0.08 **	50.36 ± 1.79 **
2	DIABETIC CONTROL	5.86 ± 0.21[a]	6.22 ± 0.22[a]	3.68 ± 0.13[a]	66.47 ± 2.36[a]
3	DIABETIC + DOSE A	1.78 ± 0.06[a]**	5.62 ± 0.20[a]**	2.77 ± 0.1[a]**	48.07 ± 1.71 **
4	DIABETIC + DOSE B	1.5 ± 0.053[a]**	4.02 ± 0.14[a]**	2.62 ± 0.09[a]**	45.4 ± 1.61[a]**
5	DIABETIC + DOSE C	2.99 ± 0.12[b]**	3.51 ± 0.11 **	2.28 ± 0.01 **	40.27 ± 1.43[a]**
6	NORMAL + DOSE A	2.76 ± 0.1 **	2.84 ± 0.10[a]**	2.17 ± 0.06 **	40.05 ± 1.42[a]**
7	NORMAL + DOSE B	2.17 ± 0.08[a]**	2.89 ± 010[a]**	1.29 ± 0.04[a]**	30.21 ± 1.07[a]**
8	NORMAL + DOSE C	2.77 ± 0.1 **	2.55 ± 0.091[a]**	1.24 ± 0.044[a]**	25.11 ± 0.89[a]**

Ethics approval and consent to participate

All the experiments were approved by the Ethics Committee of University of Calicut, Kerala, India.

Human and animal rights

No humans were used. All animal experiments were conducted in accordance with the ethical standards and national guidelines.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Funding

This work was supported by a Junior Research Fellowship to Smitha Renganathan from University of Calicut, India.

Statement

The findings presented here are not presented anywhere else for consideration. All authors have participated in the creation of this manuscript and the data underlying this. All authors had agreed to publish this article in this form in the journal Biochemistry and Biophysics Reports.

Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgements

Declared none.

References

[1] S.M. Grundy, J.I. Cleeman, S.R. Daniels, et al., Diagnosis and management of the metabolic syndrome. An American heart association/national heart, lung, and blood institute scientific statement, Circulation 112 (2005) 2735–2752.
[2] S. Shobana, Y.N. Sreerama, N.G. Malleshi, Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: mode of inhibition of aglucosidase and pancreatic amylase, Food Chem. 115 (2009) 1268–1273.
[3] P. Gerber, G.A. Rutter, The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus, Antioxidants Redox Signal. 26 (10) (2017) 501–518.
[4] C. Leloup, C. Tourrel-Cuzin, C. Magnan, et al., Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion, Diabetes 58 (3) (2009) 673–678.
[5] S. Lenzen, J. Drinkgern, M. Tiedge, Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues, Free Radic. Biol. Med. 20 (1996) 463–466.
[6] P. Newsholme, E.P. Haber, S.M. Hirabara, et al., Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity, J. Physiol. 583 (2007) 9–24.
[7] J.V. Hunt, C.C.T. Smith, S.P. Wolff, Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose, Diabetes 39 (1990) 1420–1424.
[8] P. Mohanty, W. Hamouda, R. Garg, et al., Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes, J. Clin. Endocrinol. Metab. 85 (2000) 2970–2973.
[9] B. Halliwell, Free radicals and antioxidants –quo vadis? Trends Pharmacol. Sci. 32 (2011) 125–130.
[10] A.Y. Andreyev, Y.E. Kushnareva, A.A. Starkov, Mitochondrial metabolism of reactive oxygen species, Biochemistry 70 (2005) 200–214.
[11] L.M. Yung, F.P. Leung, X. Yao, et al., Reactive oxygen species in vascular wall, Curr. Drug Targets - Cardiovasc. Hematol. Disord. 6 (2006) 1–19.
[12] L. Wu, K.G. Parhofer, Diabetic dyslipidemia, Metabolism 63 (2014) 1469–1479.
[13] C.S. Fox, S. Coady, P.D. Sorlie, et al., Trends in cardiovascular complications of diabetes, J. Am. Med. Assoc. 292 (2004) 2495–2499.
[14] J.M. Baena-Dıez, J. Penafiel, I. Subirana, et al., Risk of cause specific death in individuals with diabetes: a competing risks analysis, Diabetes Care 39 (11) (2016) 1987–1995.
[15] Y. Zeng, S.H. Ley, F.B. Hu, Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, Nat. Rev. Endocrinol. 14 (2018) 88–98.
[16] N.H. Cho, J.E. Shaw, S. Karuranga, IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045, Diabetes Res. Clin. Pract. 138 (2018) 271–281.
[17] J.K. Grover, S. Yadav, V. Vats, Medicinal plants of India with anti-diabetic potential, J. Ethnopharmacol. 81 (2002) 81–100.
[18] S.K. Swanston-Flatt, P.R. Flatt, C. Day, et al., Traditional dietary adjuncts for the treatment of diabetes mellitus, Proc. Nutr. Soc. 50 (1991) 641–651.
[19] Y.N. Upadhyaya (Ed.), Ashtanga Hridaya of Vagbhata, Shareera Sthanam; Garbhavyapad, Chowkhambha Sanskrit Publishers, Varanasi, 2006.
[20] S.R. Warrier, N. Haridas, S. Balasubramanian, et al., A synthetic formulation, Dhanwantharam kashaya, delays senescence in stem cells, Cell Prolif 46 (3) (2013) 283–290.
[21] C.V. Sruthi, A.A. Sindhu, Comparison of the antioxidant property of five Ayurvedic formulations commonly used in the management of vatavyadhis, J. Ayurveda Integr. Med. 3 (2012) 29–32.
[22] O. Yildirim, The effect of vitamin C and cobalt supplementation on antioxidant status in healthy and diabetic rats, Afr. J. Biotechnol. 8 (2009) 5053–5058.
[23] P. Kakkar, B. Das, P.N. Viswananthan, A modified spectrophotometric assay of superoxide dismutase, Indian J. Biochem. Biophys. 2 (1984) 130–132.
[24] A.C. Maehley, B. Chance, The assay of catalases and peroxidases, Methods Biochem. Anal. 1 (1954) 357–424.
[25] N. Agerguard, P.J. Jense, Procedure for blood glutathione peroxidase determination in cattle and swine, Acta Vet. Scand. 23 (1982) 515–516.
[26] M. David, J.S. Richard, H. Bergmeyer, UlrichJr. (Eds.), Methods of Enzymatic Analysis, vol. 3, 1983, p. 258.
[27] B. Zak, R.C. Dickenman, E.G. White, Rapid estimation of free and total cholesterol, Am. J. Clin. Pathol. 24 (1954) 1307–1315.
[28] K. Falholt, B. Lund, B. Falholt, An easy colorimetric micro method for routine determination of free fatty acids in plasma, Clin. Chem. Acta. 46 (2) (1973) 105–111.
[29] B.B. Zilversmith, A.K. Davis, Micro determination of plasma phospholipids by trichloroacetic acid precipitation, J. Lab. Clin. Med. 35 (1957) 155–160.
[30] E. Van Handel, D.B. Zilversmit, S. Frankel, S. Reitman, A.C. Sonnenwrith (Eds.), Serum Triglycerides Micro Method in Gradwohl's Clinical Laboratory Methods and Diagnosis, The CV mosby company, Saint Louis, 2003, pp. pp263–271.
[31] G. Drews, P. Krippeit-Drews, M. Dufer, Oxidative stress and beta-cell dysfunction, Pflügers Archiv 460 (2010) 703–718.
[32] M. Comporti, Lipid peroxidation and cellular damage in toxic liver injury, Lab. Invest. 53 (6) (1985) 599–623.
[33] D. Del Rio, A.J. Stewart, N. Pellegrini, A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress, Nutr. Metabol. Cardiovasc. Dis. 15 (4) (2005) 316–328.
[34] V.T. Selvan, L. Manikandan, G.P.S. Kumar, et al., Antidiabetic and antioxidant effect of methanol extract of Artanema sesamoides in streptatozocin-induced diabetic rats, Int. J. Appl. Res. Nat. Prod. 1 (1) (2008) 25–33.
[35] M.S. Sreekutty, S. Mini, Ensete superbum ameliorates renal dysfunction in experimental diabetes mellitus, Iran J. Basic Med. Sci. 19 (2016) 111-118.

[36] A. Negre-Salvayre, R. Salvayre, N. Auge, et al., Hyperglycemia and glycation in diabetic complications, Antioxidants Redox Signal. 11 (2009) 3071–3109.
[37] I.A. Iwara, E.A. Otu, E.E. Efiong, et al., Evaluation of the nephroprotective effects of combined extracts of Vernonia amygdalina and Moringa oleifera in diabetes induced kidney injury in albino Wistar rats, Scholars J. Appl. Med. Sci. 1 (2013) 881–886.
[38] C. Michiels, M. Raes, O. Toussaint, et al., Importance of Se-glutathione peroxidase, catalase and Cu/Zn-SOD for cell survival against oxidative stress, Free Radical Biol. Med. 17 (1994) 235–248.
[39] M.C. Deeds, J.M. Anderson, A.S. Armstrong, et al., Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models, Lab. Anim 45 (3) (2011) 131–140.
[40] M. Jayachandran, R. Vinayagam, R. Ambati, et al., Guava leaf extract diminishes hyperglycemia and oxidative stress, prevents β-cell death, inhibits inflammation, and regulates NF-kB signaling pathway in STZ induced diabetic rats, BioMed Res. Int. (2018) 4601649, https://doi.org/10.1155/2018/4601649 eCollection 2018 pp. 14.
[41] O. Coskun, M. Kanter, A. Korkmaz, et al., Quercetin, a flavonoids antioxidant, prevents and protects streptozotocininduced oxidative stress and b-cell damage in rat pancreas, Pharma Res. 51 (2005) 117–123.
[42] M. Hao, W.S. Head, S.C. Gunawardana, et al., Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction, Diabetes 56 (2007) 2328–2338.
[43] T. Aluwong, J.O. Ayo, A. Kpukple, et al., Amelioration of hyperglycaemia, oxidative stress and dyslipidaemia in alloxan-induced diabetic wistar rats treated with probiotic and vitamin C, Nutrients 8 (5) (2016) E151, https://doi.org/10.3390/nu8050151.
[44] H. Yadav, S. Jain, P. Sinha, Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats, Nutrients 23 (2007) 62–68.
[45] A.S. Matheus, L.R. Tannus, R.A. Cobas, et al., Impact of diabetes on cardiovascular disease: an update, Int. J. Hypertens. (2013) e653789, , https://doi.org/10.1155/2013/653789.
[46] A.K. Fenercioglu, T. Saler, E. Genc, et al., The effects of polyphenol-containing antioxidants on oxidative stress and lipid peroxidation in type 2 diabetes mellitus without complications, J. Endocrinol. Invest. 33 (2010) 118–124.
[47] I.S. Stephen, C. Sunil, V. Duraipandiyan, et al., Antidiabetic and antioxidant activities of toddalia asiatica (L.) lam. Leaves in streptozotocin induced diabetic rats, J. Ethnopharmacol. 143 (2012) 515–523.
[48] S.Y. Park, S.H. Bok, S.M. Jeon, et al., Effect of rutin and tannic acid supplements on cholesterol metabolism in rats, Nutr. Res. 22 (2002) 283–295.