International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN

Original Research Article

Comparative anti-diabetic study of three phytochemicals on high-fat diet and streptozotocin-induced diabetic dyslipidemic rats

Chetna Mishra[1,2*], M.A. Khalid[2], Dinesh Tripathi[3] and A. A. Mahdi[1]

[1]*Department of Biochemistry, King George's Medical University, Lucknow, India-226003*
[2]*Department of Environmental Science, Integral University, Lucknow, India-226026*
[3]*Department of Physiology, King George's Medical University, Lucknow, India-226003*

QR Code

***Correspondence Info:**
Ms. Chetna Mishra
Department of Biochemistry,
King George's Medical University,
Lucknow, Uttar Pradesh, India- 226003

***Article History:**
Received: 28/07/2018
Revised: 10/08/2018
Accepted: 11/08/2018
DOI: https://doi.org/10.7439/ijbar.v9i8.4862

Abstract

Objective: Phytochemicals which are derived from medicinal plants having properties to cure the early stage of diabetes and its complications according to various preliminary studies. Mangiferin, Camphene and Citral are a major chemical component of *Mangifera indica* (mango), *Zingiber officinale* (ginger) and *Cymbopogon citratus* (lemongrass), respectively has been reported multiple biological activities like antioxidant, anti-inflammatory, antidiabetic antidyslipidemic, antimicrobial, antitumor etc. Aim of this study was to compare the antidyslipidemic, antidiabetic as well as antioxidant effects of Mangiferin, Camphene and Citral on streptozotocin (STZ) + high-fat diet (HFD) induced diabetic dyslipidemic rats.

Material & Methods: Diabetes was induced by through an STZ at a dose of 35 mg/kg/b.w injected intraperitoneally (i.p.). Plasma insulin and Glycosylated Haemoglobin (HbA1c) were estimated by kits. Free Fatty Acids (FFA), Triglycerides (TG), Phospholipids, Small dense lipoproteins (s-LDL) and total cholesterol (TC) were also estimated in blood serum by using kits. Other biochemical parameters like Thiobarbituric acid reactive substances (TBARS), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione Reductase (GR) and reduced Glutathione (GSH) were estimated in the pancreas, skeletal muscles and adipose tissue homogenates spectrophotometrically.

Results: Citral has shown more degree of anti-hyperglycemic activity and antioxidant activity than Mangiferin and Camphene do. But, all three have produced a similar degree of hypolipidemic activity.

Conclusion: Current study shows that Mangiferin, Camphene and Citral possess significant antidiabetic, antidyslipidemic and antioxidant properties thus it will provide new direction in the herbal treatment of diabetic dyslipidemia and related cardiovascular complications**.**

Keywords: Diabetes; Dyslipidemia; Mangiferin; Citral; Camphene; Oxidatives-Stress.

1. Introduction

Medicinal plants are a rich source of bioactive phytochemicals which have the potential for preventing chronic disease such as cancer, Alzheimer, diabetes, cardiovascular disease etc. Several phytochemicals including alkaloids, flavonoids, glycosides, glycolipid, polysaccharides, peptidoglycans, carbohydrates, amino acids and saponin obtained from plant sources have been reported to possess antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, antidyslipidemic activities etc.

Diabetes mellitus, is a major global problem, millions of people suffers from diabetes in the whole world. It is characterized by chronic hyperglycaemia (high blood sugar) with disturbances of carbohydrate, fat and protein metabolism [1]. Hyperglycemia is known to produce reactive oxygen species (ROS) which react with lipids, initiating their peroxidation leading to abnormalities in lipid metabolism and dyslipidemia in diabetes mellitus [2]. Thus oxidative load lipotoxicity as well as hyperglycemia-mediated glucotoxicity in diabetic dyslipidemia may causes complications in diabetes [3].

The association of dyslipidemia and oxidative stress may accelerate the dysfunctions in beta cells and thus diabetic complications like retinopathy, nephropathy, neuropathy, cardiopathy and many more [4]. The key feature of diabetic dyslipidemia is the elevation of serum total cholesterol (TCh), triglycerides (TG) and low-density lipoprotein (LDL) levels associated with a reduced high density lipoprotein (HDL) cholesterol [5].

Treatment of patients with diabetes includes balanced diet, exercise, oral hypoglycemic agents, and / or insulin [6,7]. Hypoglycemic agents which are currently being used are expensive and also have side effects. Therefore, herbal medicines recommended for treatment of diabetes because of their effectiveness, less side effects and relatively low cost [8, 9].

Mangiferin ($C_{19}H_{18}O_{11}$) a glucoxanthone, is a major chemical component of *Mangifera indica* has been reported multiple biological activities [10]. A major component of *Cymbopogan citratus* is a Citral, which is a mixture of two terpenoids geranial and neral with the molecular formula $C_{10}H_{16}O$ [11]. Camphene is bicyclic monoterpens and present in essential oil of ginger (*Zingiber officinale*), tulsi (*Ocimum sanctum*) etc [12]. There are some preliminary reports on Mangiferin from mango (*Mangifera indica* L), Citral from lemongrass (*Cymbopogon citratus* (DC.) Stapf), to possess antioxidant, antitumor, chemopreventive, antimicrobial, anti-inflammatory, antidiabetic, antidyslipidemic etc, but no in depth study has been done so far. Here, the present work is design to compare the antidyslipidemic, antioxidant and antidiabetic effects of Mangiferin, Camphene and Citral on high fat diet (HFD) and low dose streptozotocin (STZ) induced diabetes dyslipidemia on wistar rats.

2. Materials and Methods

2.1 Chemicals and reagents:

Trichloroacetic acid (TCA), Glutathione reduced (GSH), 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), ethylene diamine tetra acetic acid (EDTA) and 2, 4-dinitrophenyhydrazine (DNPH), streptozotocin (STZ), Citral were purchased from Sigma–Aldrich, Chemicals Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

2.2 Animals

Male albino Sprague–Dawley rats weighing 200-250 gm were used in this study. All rats were kept at room temperature of 20°C in the central Animal house of the Medical College. They were maintained on Standard diet-pellets (Ashirvad Industries, Chandigarh) and water *ad libitum*. All experiments were performed as per the directives of the institutional Animal Ethics Committee (40/IAEC/2013).

2.3 Experimental design and development of HFD/STZ model of type 2 diabetes:

In this study we induced diabetes mellitus by administration of low dose of streptozotocin in HFD fed rats according to the method of Srinivasan *et al* [13]. Male Sprague–Dawley rats (200-250 g) were fed with HFD for 2 weeks. After 2 weeks of dietary manipulation, they were injected intraperitoneally a single dose of streptozotocin (35 mg /kg) dissolved in 50m M cold citrate buffer (pH 4.5).The feeding with HFD were continue till the end of experiment. The blood glucose values above 180mg/dl on the third day after STZ injection were considered as diabetic rats.

2.4 Treatment Schedule

Mangiferin, Camphene Citral, as well as Fenofibrate and Glibenclamide were macerated with in tripled distilled water containing 2% gum acacia and fed orally once daily by gastric tube at the doses mentioned below. The study was comprised of the following groups

Group-I : Normal rats fed on saline (control)
Group-II : HFD/STZ treated rats fed on saline (Diabetic Control)
Group-III : HFD/STZ treated rats fed on Mangiferin (45mg/kg b.w./daily)
Group-IV : HFD/STZ treated rats fed on Camphene (45mg/kg b.w./ daily)
Group-V : Diabetic rats fed on *Citral* (45 mg/kg b.w./day)
Group-VI : Diabetic rats fed on Glibenclamide (600µg/ kg b.w.)
Group-VII : Diabetic rats fed on Fenofibrate once daily (500mg/kg b.w.)

2.5 Oral glucose tolerance test (OGTT)

Two days before termination of experiment, the OGTT was performed to assess the glucose tolerance. For this purpose, overnight fasted rats were fed orally 2 gm/kg body wt glucose. Blood was collected at 0, 30, 60 and 120 min intervals from orbital sinus for glucose estimation. Animals were not anesthetized for this procedure.

2.6 Tissue preparation

At the end of experiment, rats were anesthetized by ether inhalation and blood was collected from the dorsal aorta. Rats were then sacrificed and their liver, adipose tissues, and pancreas were excised immediately and perfused with ice-cold saline. For biochemical estimations, all the tissues were homogenized at 4°C with 10 times (w/v) 0.1 M phosphate-buffer (pH 7.4) containing protease inhibitors in a tissue homogenizer (Kinematica A.G.). The homogenate was centrifuged at 800 x *g* for 5 min at 4°C to separate the nuclear debris and was used for estimation of thiobarbituric reactive substances (TBARS).

The supernatant was further centrifuged at 10,000 x *g* for 20 min at 4°C to get the post-mitochondrial supernatant (PMS), which was used for various biochemical assays. Samples were stored at -80°C before analysis [14].

2.7 Estimation of Plasma glucose and insulin level:

To estimate blood glucose level by the method of Trinder [15]. Fasting plasma insulin level was measured by ELISA kit. [16]. The level of glycosylated proteins O_2 haemoglobin was estimated by thiobarburic acid reaction according to method of Goldstein *et al* [17] and protein was estimated by Lowry *et al* [18]

2.8 Assessment of Dyslipidemic Parameters.

Quantitation of small dense LDL was measured by Okada *et al* [19]. Free Fatty Acid estimation was determined by the method Mosinger [20], Total cholesterol was measured by the method of Deeg & Ziegenborn [21]. Phospholipids measured by Kallner) [22] and triglycerides were measured by Buccolo & David [23].

2.9 Assessment of Oxidative-Antioxidative Status in Pancreas, Skeletal muscles and Adipose tissues

Lipid peroxidation level was determined according to the method Ohkhawa and Ohishi [24] and Xanthine Oxidase activity was determined by [25] Haideri *et al* in all the tissues. Protein carbonyl groups were measure by spectrophotometric method with the use of the carbonyl specific reagent dinitrophenylhydrazine (DNPH). The optical density of which was read in UV range at 280 nm on spectrophotometer [26].

SOD assay was assayed by the McCord and Fridovich [27]. The absorbance will be read at 560 nm against blank. Catalase was assayed by following the method Aebi [28]. GPx was determined by the method Pagilia and Valentine [29]. Enzyme unit was defined as nmole of NADPH oxidized per minute per mg protein. GR was estimated by follow the method of Hazelton and Lang [30]; results were expressed as unit/min/mg protein. Reduced glutathione was estimated by the method of Ellman [31] in all tissues.

2.10 Statistical analysis

Data are expressed as mean ± SE. Data was analyzed on Graph pad Prism 5 software using student's t-test and one-way ANOVA (Analysis of variance). *P < 0.05, **P < 0.01, ***P < 0.001 were used as the criterion for significance.

3. Results

3.1 Effect of all three phytochemicals on diabetic parameters

Blood glucose levels of all the groups at different time points (0, 30, 60, 90 and 120 mins) after the oral administration of glucose (2gm/kg) have shown in table 1. In the diabetic control (Group-II) rats the significant increase in blood glucose level was observed after 60 mins and remained high to next 60 mins. Among all the three phytochemicals Citral (Group-V) administered group showed significant decrease ($p<0.01$) in blood glucose levels at 90, and 120 mins as equal to Glibenclamide (Group-VI) administered group. Table 2 showed a serum glucose and HbA1C level of the diabetic group were higher and insulin level was lower than in the non-diabetic group, indicating a sustained hyperglycemic state in the STZ-induced diabetic rats. Administration of Mangiferin (Group-III), Camphene (Group-IV) were significant ($p< 0.05$), whereas Citral (Group-V) was highly significant ($p<0.001$) reduced blood glucose and HbA1C level as compared to diabetic control rats. It has been observed that all the phytochemicals were significantly improve insulin level like Glibenclamide (Group-VI) does. Fenofibrate (Group-VII) treated group did not show any significant changes in all the parameters.

3.2 Effect of Phytochemicals on Lipid profiles of diabetic dyslipidemic rats

Table 3 showed effect of Mangiferin, Camphene and Citral on lipid profile of diabetic rats. The diabetic control group showed a significant ($p < 0.01$) increment in serum TC, TG, FFAs, s LDL, phospholipids levels ($p < 0.05$) compared to the control group (Table 3). Treatment of Mangiferin, Camphene and Citral group significantly ($p < 0.05$) restored all the changes in lipid profile and FFAs compared to the HFD/STZ group. Fenofibrate treated group showed highly significant ($p<0.001$) decrease in TC, TG, FFA's, sLDL and phospholipids level.

3.3 Effect of phytochemicals on oxidative Stress

Effect of Mangiferin, Camphene and Citral on lipid peroxidation, Xanthine oxidase and protein carbonyl was measured in the pancreas, skeletal muscles and adipose tissue in the HFD/STZ induced diabetic dyslipidemic rats. MDA, XO and PC (Figure 1) level were significantly ($P< 0.05$) increased in all selected tissues of diabetic dyslipidemic rats as compared to normal control rats (Group-I).

However, among the all three phytochemicals, a significant reduction in the level of MDA, XO and PC were observed in the Citral treated groups as equal to Glibenclamide does. Streptozotocin injection significantly ($P<0.001$) decreased the activity of SOD, Catalase, GPx, GR and GSH in all selected tissues of diabetic rats as compared to control rats. Supplementation of Mangiferin, Camphene improved significant (0.05) and Citral also improved highly significant enzyme activity in diabetic dyslipidemic rats (Figure 2 and Figure 3)

Table 1: Effect of phytochemicals on Oral Glucose Tolerance

Group	Glucose (mg/dl) at different time intervals				
	0 min	30 min	60 min	90 min	120 min
Group-I	94±53.2	110±36.41	115±21.69	110±51.51	86±49.32
Group-II	402±48.6#	415±94.21#	425±86.31#	435±86.95#	430±95.36#
Group-III	175±74.36	180±86.47	198±74.21	187±87.14	180±74.26
Group-IV	180±86.21	189±56.87	205±72.68	195±74.58	192±54.39
Group-V	150±65.34	155±36.47	170±64.75	160±56.47**	145±86.14**
Group-VI	140±81.23	150±75.6	162±56.25	145±56.28**	130±88.24**
Group-VII	210±97.21	224±84.25	230±86.2	232±74.36	210±87.25

Values are expressed as mean± S.D. # denotes significant difference compared with control rats; * P < 0.05; ** P < 0.01; *** P < 0.001 denotes significant difference compared with diabetic control

Table 2: Effect of Mangiferin, Citral and Camphene on blood glucose, HbA1C and insulin

Groups	Blood glucose level (mg/dl)	Insulin (µU/ml)	Glycosylated haemoglobin (%Hb)
Group-I	110.6±26.96	17.54±5.42	4.2±2.74
Group-II	320.8±81.66#	6.45±3.68#	13.70±3.34#
Group-III	158.8±47.27*	12.77±4.27*	8.24±2.74*
Group-IV	169.8±37.61*	10.85±6.74	8.93±2.41*
Group-V	143±26.59***	13.12±7.78**	7.19±2.83**
Group-VI	132±26.83***	16.10±4.06**	5.94±1.58**
Group-VII	206±59.41	8.92±4.01	10.55±4.20

Values are expressed as mean± S.D. # denotes significant difference compared with control rats; * P < 0.05; ** P < 0.01; *** P < 0.001 denotes significant difference compared with diabetic control

Table 3: Effect of Mangiferin, Citral and Campene on lipid profiles and Free Fatty acid

Group	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	Free fatty acids (mg/dl)	Phospholipids (mg/dl)	Low Dense lipoproteins (mg/dl)
Group-I	86±36.64	44.4±15.26	64±20.73	105±25.49	77±29.70
Group-II	266±74.36 #	104±51.28 #	203±75.13#	216±59.41#	240±87.10#
Group-III	187.6±73.86	85±19.03	143.8±22.77	178±79.34	195±65.09
Group-IV	199±78.05	90.4±30.18	146±36.29	189±91.45	193.4±63.24
Group-V	136.06±25.01*	82.6±25.00*	130.4±35.99*	128.6±32.01*	122.6±37.55
Group-VI	188.6±68.64	81.6±22.14	126.8±28.7	168±88.84	120.75±86.14
Group-VII	126.8±51.22***	70.2±21.74***	106±25.59***	124.6±41.19***	106.2±56.50***

Values are expressed as mean± S.D. # denotes significant difference compared with control rats; * P < 0.05; ** P < 0.01; *** P < 0.001 denotes significant difference compared with diabetic control

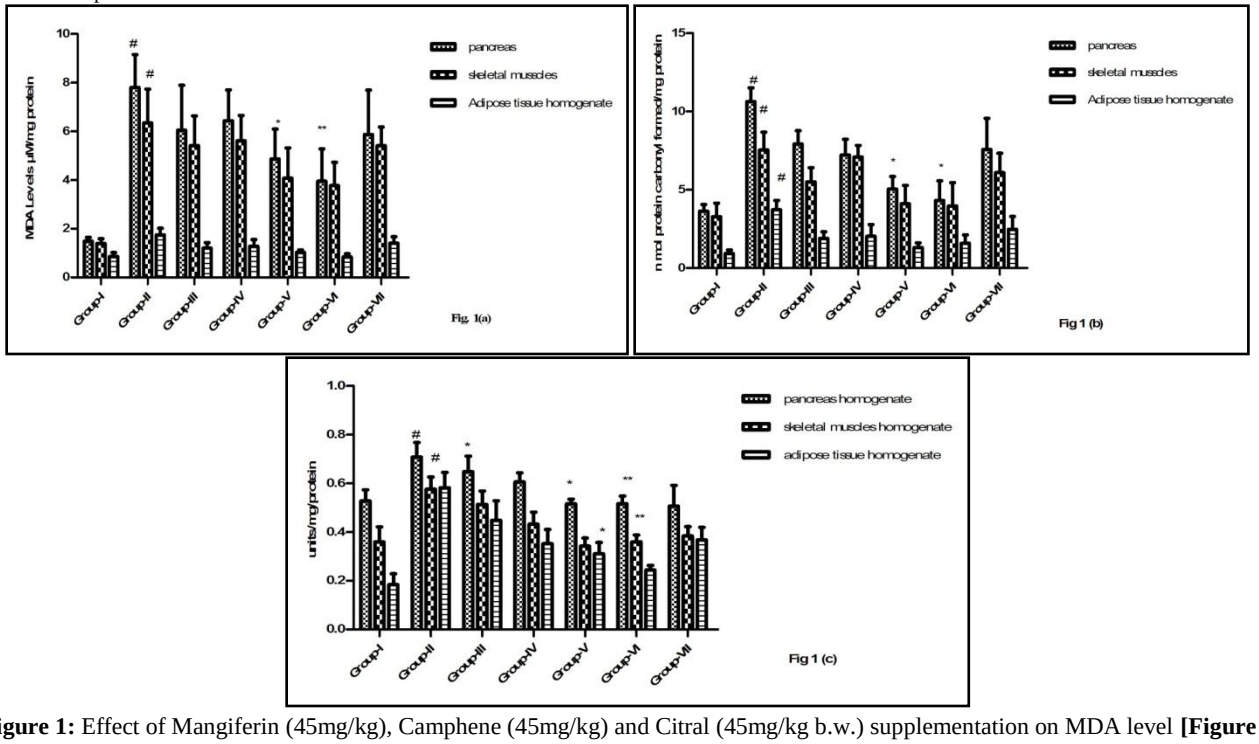

Figure 1: Effect of Mangiferin (45mg/kg), Camphene (45mg/kg) and Citral (45mg/kg b.w.) supplementation on MDA level **[Figure 1 (a)]**, protein carbonyl content **[Figure 1 (b)]** and Xanthine Oxidase activity **[Figure 1 (c)]** of STZ + HFD induced diabetic dyslipidemic rats. (No. of rats per group=6). # denotes significant difference compared with control rats; * P < 0.05; ** P < 0.01; *** P < 0.001 denotes significant difference compared with diabetic control. **Group I**-control; **Group II**- diabetic control, **Group III**- Mangiferin treated rats; **Group IV**- Camphene treated rats; **Group V**- Citral treated rats; **Group VI** -Glibenclamide treated rats; **Group VII**- Fenofibrate treated rats

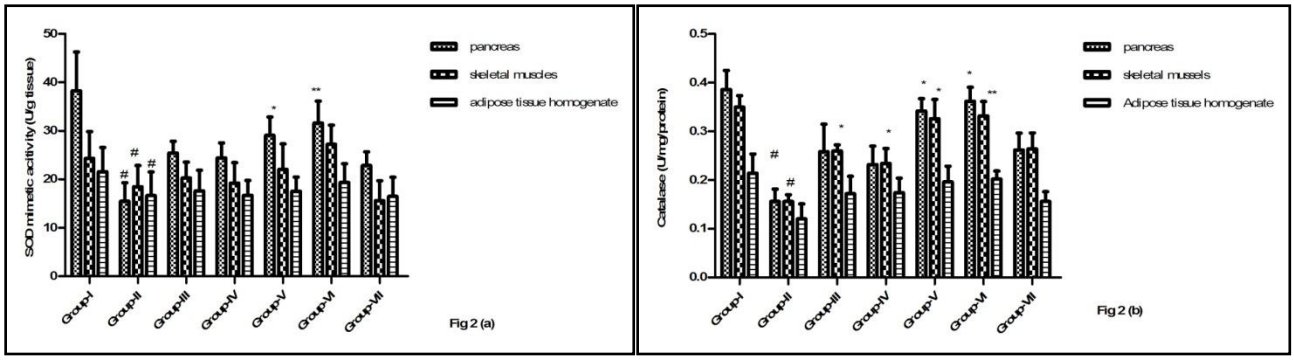

Figure 2: Effect of Mangiferin (45mg/kg), Camphene (45mg/kg) and Citral (45mg/kg b.w.) supplementation on SOD mimetic activity **[Figure 2 (a)]** and Catalase activity **[Figure 2 (b)]** of STZ + HFD induced diabetic dyslipidemic rats. (No of rats per group=6) # denotes significant difference compared with control rats; * P < 0.05; ** P < 0.01; *** P < 0.001 denotes significant difference compared with diabetic control. **Group I**-control; **Group II**- diabetic control, **Group III**- Mangiferin treated rats; **Group IV**- Camphene treated rats; **Group V**- Citral treated rats; **Group VI**- Glibenclamide treated rats; **Group VII**- Fenofibrate treated rats

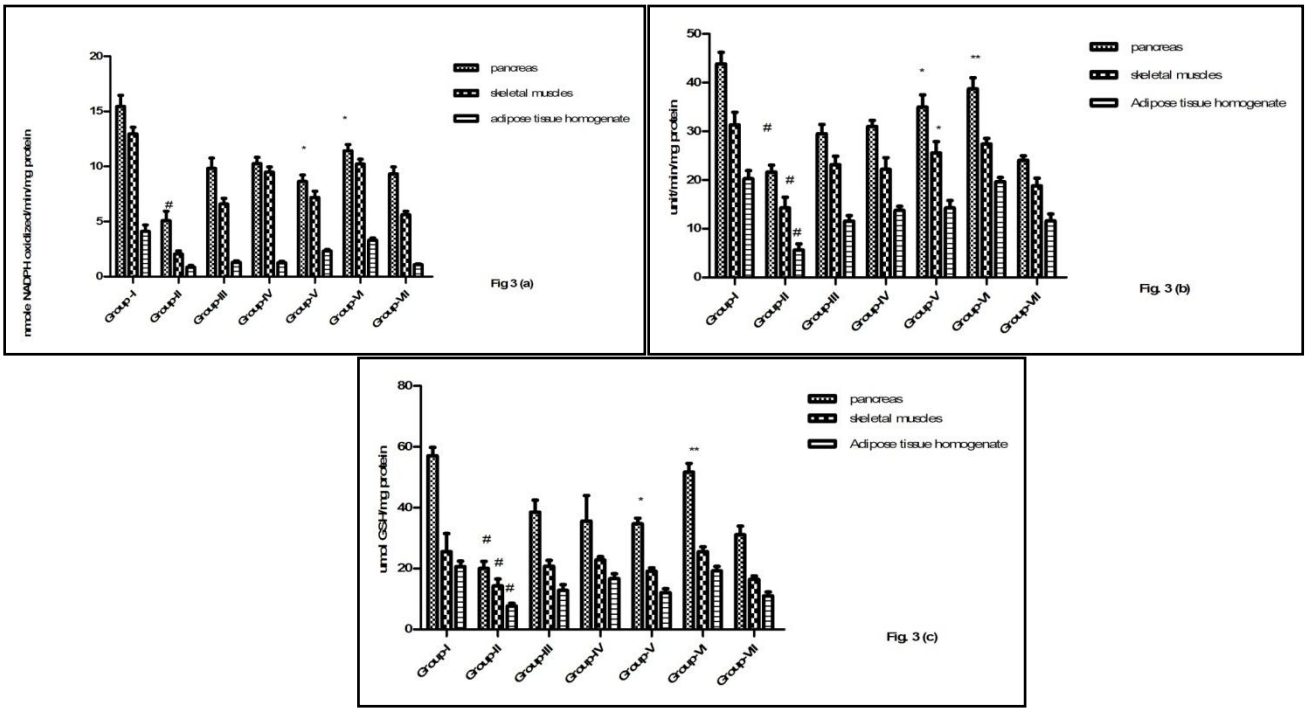

Figure 3: Effect of Mangiferin (45mg/kg), Camphene (45mg/kg) and Citral (45mg/kg b.w.) on GPx activity **[Figure 3 (a)],** GR activity **[Figure 3 (b)]** and GSH activity **[Figure 3 (c)]** of STZ + HFD induced diabetic dyslipidemic rats. (No of rats per group=6) # denotes significant difference compared with control rats; * P < 0.05; ** P < 0.01; *** P < 0.001 denotes significant difference compared with diabetic control. **Group I**-control; **Group II**- diabetic control, **Group III**- Mangiferin treated rats; **Group IV**- Camphene treated rats; **Group V**- Citral treated rats; **Group VI** -Glibenclamide treated rats; **Group VII**- Fenofibrate treated rats

4. Discussion

Dyslipidemia is common characteristic feature of Type 2 diabetes with elevated triglyceride (TG), total cholesterol, low-density lipoprotein cholesterol (LDL-c), and decreased high-density lipoprotein cholesterol, which are partly responsible for the contribution to insulin resistance and the development of type 2 diabetes and also a risk factor for cardiovascular disease (CVD) [32-34]. Therefore, intensive lipid-lowering therapy should be used for primary and secondary preventive therapy against macrovascular complications in Type 2 diabetic patients [35]. Several reports indicate that herbal medicines possess hypoglycemic and hypolipidemic property [36-38]. Hence in the present study, we compare Lipid and Glycemic Effects of Mangiferin, Citral and Camphene on high-fat diet and low-dose streptozotocin-induced diabetic dyslipidemia on rats.

The present study demonstrates that the effect of phytochemicals on high-fat diet for 4 weeks combined with low-dose streptozotocin induced diabetes dyslipidemia. Type 2 diabetic control rats showed significant hyperglycemia after 3 days of STZ treatment. Development of glucose intolerance in HFD combined with low-dose STZ-induced diabetic rats was found in the literature [39,40]. Here, in the present study, we investigate the anti-hyperglycemic effect of all the three phytochemicals

through the oral glucose tolerance test. The OGT test showed that the Citral decreases the glucose and increase the body weight more effectively than Mangiferin and Camphene treated groups and significantly improve glucose tolerance, because of Citral may enhance glucose utilization compare to other two phytochemicals. These results were similar to earlier studies on medicinal plants extract or phytochemicals [41,42]. Insulin levels were significantly increased in both Mangiferin and Camphene treated group but highly significant increased level of insulin in Citral treated group were found. This results indicated that Citral stimulate insulin secretion from beta cells or regenerate beta cells. Glycosylated hemoglobin was found to increase in the diabetic rats and the amount of increase is directly proportional to the fasting blood glucose level [43,44]. Administration of Citral, Camphene and Mangiferin prevents a significant elevation in Glycosylated hemoglobin thereby increasing the level of total hemoglobin in diabetic rats as reported by earlier findings [45].

As we know that dyslipidemia is linked with diabetes mellitus. It has been demonstrated that during DM a variety of derangements in metabolic and regulatory processes occurs, which in turn leads to hyperlipidemic condition in diabetic people [46]. The plasma levels of TC, LDL and TG increase while HDL level decrease contributing the diabetic dyslipidemic condition. In diabetes shortage of insulin initially causes an increase in free fatty acid mobilization from adipose tissue [47]. Increased level of free fatty acid might induce lipotoxicity in obesity and this has been shown to be manifested by several tissues like pancreas, adipose, liver etc [48]. This study show that the decrease in plasma glucose, triglycerides, total cholesterol, and LDL with a associated increase in plasma HDL in HFD/STZ induced diabetic rats in treatment of the diabetic rats with Mangiferin, and Camphene and marked decrease in Citral treated group. Similar effects of Mangiferin in improving dyslipidemia were previously reported in streptozotocin diabetic rats and similar results of this model have been explained earlier by many reports [49,50]. The presently observed decline in plasma lipid profiles in all the three phytochemicals administered diabetic rats suggests that it is possibly due to the elevation of insulin level.

Reactive oxygen species and their role in the generation and progression of diabetes have been reported by many researchers. In the current investigation we also estimated the levels of antioxidant enzymes and lipid peroxidation in STZ and HFD diet induced diabetes dyslipidemia in rats.

The results of this study showed a marked reduction in antioxidant enzymes including SOD, GPx and CAT in diabetic animals with decreased GSH and increased MDA and XO levels indicating oxidative stress. Free radicals generated due to different glucose metabolizing pathways can be well nullified by phytochemicals having good antioxidant property [51,52]. All the three phytochemicals were able to restore the modulation seen in SOD, Catalase, GPx activity and redox ratio due to diabetes induction. Moreover, Citral showed significant reduction of lipid peroxidation, XO and protein carbonyl content in diabetic rats. Our results on the disturbed antioxidant status due to diabetes are support the earlier findings reporting the use of phytochemicals in effective treatment of tissue damage during diabetes [53-55]. The curative and preventive property of Citral in diabetic rats may be due to its improvement in glucose tolerance and antioxidant property.

5. Conclusion

The data of our study shows that Citral possesses significant antidiabetic as well as antidyslipidemic property than Camphene and Mangiferin. Results of this study also indicate that the protective effect of Citral in hyperglycemic conditions may be associated with an increase of glucose uptake by peripheral tissues. In conclusion, since Citral possesses significant hypoglycemic potentiality, it may be used for the development of antidyslipidemic drug in diabetes. However, depth studies will be needed to explain more fully mechanism(s) of antidiabetic as well as antidyslipidemic effects of Citral.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are grateful to Indian Council of Medical Research, New Delhi for giving fellowship and financial support and are also grateful to the Vice-chancellor and publication review committee of Integral University for allocating the manuscript number (IU/R&D/2017-MCN000186)'

References

[1]. Gaballu FA, Gaballu YA, Khyavy OM, Mardomi A, Ghahremanzadeh K, Shokouhi B, *et al*. Effects of a triplex mixture of *Peganum harmala*, *Rhuscoriaria*, and *Urticadioica* aqueous extracts on metabolic and histological parameters in diabetes rats. *Pharm Biol* 2015; 53:1104- 9.

[2]. Shengule SA, Mishra S, Joshi K, Apte K, Patil D, Kale P, Shah T, Deshpande M, Puranik A. Anti-hyperglycemic and anti-hyperlipidaemic effect of Arjunarisht in high-fat fed animals. *J Ayurveda Integr Med* 2017; 15. Doi: 10.1016/j.jaim.2017.07.004

[3]. Vanessa Fiorentino T, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. *Current Pharma Design* 2013; 19(32): 5695-703.

[4]. Chandra A, Mahdi AA, Singh RK, Mahdi F, Chander R. Effect of Indian herbal hypoglycemic agents on antioxidant capacity and trace elements content in diabetic rats. *J Med food* 2008; 11(3): 506-12.

[5]. Anwer T, Sharma M, Pillai KK, Khan G, Safhi MM. Antihyperglycemic, antidyslipidemic and antioxidant activity of *Rhus coriaria* in STZ- induced type 2 diabetic rats. *Afr J Pharm Pharmacol* 2012; 6(41): 2851-5.

[6]. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J Diabetes* 2015; 6(3): 456.

[7]. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, *et al*. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. *Diabetes care* 2009; 32(1): 193-203.

[8]. Pfeiffer AF, Klein HH. The treatment of type 2 diabetes. *Dtsch Arztebl Int* 2014; 111(5): 69.

[9]. Gothai S, Ganesan P, Park SY, Fakurazi S, Choi DK, Arulselvan P. Natural phyto-bioactive compounds for the treatment of type 2 diabetes: inflammation as a target. *Nutrients* 2016; 8(8): 461.

[10]. Muruganandan S, Srinivasan, K, Gupta S, Gupta PK, Lal J. Effect of Mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. *J Ethnopharmacol* 2005; 97: 497–501.

[11]. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (Lemon grass). *J Adv Pharma Technol Res* 2011; 2(1): 3.

[12]. El-Ghorab AH, Nauman M, Anjum FM, Hussain S, Nadeem M. A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). *J Agri Food Chem* 2010; 58(14): 8231-7.

[13]. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacol Res* 2005; 52: 313-320.

[14]. Maheswari R, Vennarasi M. Hypoglycemic antioxidant potential of herbal extract studied in high fat fed and low dose streptozotocin induced type 2 diabetic rats. *In Int Conf Bioinform Biomed Eng* 2015; 1: 85-92.

[15]. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969; 6: 24-27.

[16]. Grassi J, Pradelles PH. Compounds labeled by the acetyl cholinesterase of Electrophorus Electricus Its preparation process and it use as a tracer or marquer in enzymo-immunological determinations. United States patent, N°1,047; 330: 1991.

[17]. Goldstein DE, Parker KM, England JD. Clinical applications of glycosylated hemoglobin measurements. *Diabetes* 1982; 31: 70-78.

[18]. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-275.

[19]. Okada M, Mitsui H, Ito Y, Fuziwara A, Inano K. Low density lipoprotein cholesterol can be chemically measured: a new superior method. *J Lab Clin Med* 1998; 132: 195-201.

[20]. Mosinger F. Photometric adaptation of Dole's micro determination of free fatty acids. *J Lipid Res* 1965; 6: 157-159.

[21]. Deeg R, Ziegenborn J. Kinetic enzymatic method for automated determination of total cholesterol in serum. *Clin Chem* 1983; 29: 1798-1803.

[22]. Kallner AA. Direct colorimetric assay of Ca^{++} stimulated ATPase activity. *Clin Chim Acta* 1975; 59: 35-39.

[23]. Buccolo G, David H. Quantative determination of serum triglycerides by the use of enzymes. *Clin Chem* 1973; 19: 476-480.

[24]. Ohkawa H, Ohishi N. Reaction of thiobarbituric acid with linoleic acid hydroperoxide. *J Lipid Res* 1978; 19: 1053-57.

[25]. Haideri F, Rashidi MR, Keshavarz SA, Mahboob SA, Eshraghian MR, Shahi MM. Effects of onion on serum uric acid levels and hepatic xanthine dehydrogenase /xanthine oxidase activities in hyper uricemic rats. *Pak J Biol Sci* 2008; 11: 1779-1784.

[26]. Abraham ZR, Packer L. Oxidative damage to proteins: Spectrophotometeric method for carbonyl assay. *Meth Enzymol* 1994; 233: 357-363.

[27]. McCord JM, Fridovich I. Superoxide dismutase, an enzyme function for erythrocuprin (hemocuprin). *J Biol Chem* 1969; 224: 6049-6055.

[28]. Aebi H. Catalase *in vitro. Meth Enzymol* 1984; 105: 121-126.

[29]. Pagila DE, Valentine WN. Studies on the quantitation and qualitation characterization of erythrocyte glutathione peroxidase. *J Lab Clin Medicine* 1967; 70: 158-169.

[30]. Hazelton GA, Lang CA. GSH content of tissue in ageing mouse. *Biochem J* 1987; 188: 25-30.

[31]. Ellman G. Tissue sulfhydryl groups. *Arch* Biochem *Biophy* 1959; 82: 70-77.

[32]. Stahlman M, Fagerberg B, Adiels M, Ekroos K, Chapman JM, Kontush A, *et al*. Dyslipidemia, but not hyperglycemia and insulin resistance is associated with marked alterations in the HDL lipidome in type 2 diabetic subjects in the DIWA cohort: impact on small HDL particles. *Biochim Biophys Acta* 2013; 1831 (11): 1609-161.

[33]. Dickson-Humphries T, Bottenberg B, Kuntz S. Lipoprotein abnormalities in patients with type 2 diabetes and metabolic syndrome. *J Am Acad Phys Assist* 2013; 26: 13-18.

[34]. Uzzaman R, Ghaffar M. Antidiabetic and hypolipidemic effects of extract from the seed of *Gossypium herbaceum* L. in Alloxan-induced diabetic rabbits. *Pak J Pharm Sci* 2017; 30(1):75-86.

[35]. Stephen Irudayaraj S, Christudas S, Antony S, Duraipandiyan V, Naif Abdullah AD, Ignacimuthu S. Protective effects of *Ficus carica* leaves on glucose and lipids levels, carbohydrate metabolism enzymes and β-cells in type 2 diabetic rats. *Pharma Biol* 2017; 55(1):1074-1081.

[36]. Hassani MM, Ahmad A, Asmawi MZ, Mahmudi R. Preliminary investigation of normoglycemic, anti-hyperglycemic and dyslipidemic activities of different extracts of *Tinospora crispa* on diabetic rat. *Acta Pol Pharm* 2016; 73(1):129-34.

[37]. Chowtivannakul P, Srichaikul B, Talubmook C. Hypoglycemic and Hypolipidemic Effects of Seed Extract from *Antidesma bunius* (L.) Spreng in Streptozotocin-induced Diabetic Rats. Pakistan journal of biological sciences. *Pak J Biol Sci* 2016; 19(5):211-218.

[38]. Skovso S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *J Diabetes Invest* 2014; 5(4): 349-58.

[39]. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacol Res* 2005; 52: 313-320

[40]. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, *et al*. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism* 2000; 49(11): 1390–1394.

[41]. Raghavan B, Kumari SK. Effect of *Terminalia arjuna* stem bark on antioxidant status in liver and kidney of alloxan diabetic rats. *Ind J Physiol Pharmacol* 2006; 50(2):133.

[42]. Daisy P, Santosh K, Rajathi M. Antihyperglycemic and antihyperlipidemic effects of *Clitoria ternatea* Linn. in alloxan-induced diabetic rats. *Afr J Microbiol Res* 2009; 3(5): 287-91.

[43]. Eshrat HM. Effect of *Coccinia indica* (L.) and *Abroma augusta* (L.) on glycemia, lipid profile and on indicators of end-organ damage in streptozotocin induced diabetic rats. *Ind J Clin Biochem* 2003; 18(2): 54–63.

[44]. Parveen K, Khan R, Siddiqui WA. Antidiabetic effects afforded by *Terminalia arjuna* in high fat-fed and streptozotocin-induced type 2 diabetic rats. *Int J Diab. Metab* 2011; 19: 23-33.

[45]. Yusufoglu HS, Soliman GA, Abdel-Rahman RF, Abdel-Kader MS, Ganaie MA, Bedir E, *et al*. Antihyperglycemic and antihyperlipidemic effects of *Ferula duranii* in experimental type 2 diabetic rats. *Int J Pharmacol* 2015; 532-41.

[46]. Schuster DP. Obesity and the development of type 2 diabetes: the effects of fatty tissue inflammation. *Diabetes Metab Syndr Obes* 2010; 3: 253

[47]. Arvind K, Pradeepa R, Deepa R, Mohan V. Diabetes and coronary artery disease. *IJMR* 2002; 116: 121-32.

[48]. Yang H, Li X. The role of fatty acid metabolism and lipotoxicity in pancreatic *β*-cell injury: Identification of potential therapeutic targets. *Acta Pharma Sinica B* 2012; 2(4): 396-402.

[49]. Nirmala A, Saroja S, Vasanthi HR, Lalitha G. Hypoglycemic effect of *Basella rubra* in streptozotocin induced diabetic albino rats. *J Pharma Phyto* 2009; 1(2): 25-30.

[50]. Suanarunsawat T, Ayutthaya WD, Thirawarapan S, Poungshompoo S. Anti-oxidative, anti-hyperglycemic and lipid-lowering effects of aqueous extracts of *Ocimum sanctum* L. leaves in diabetic rats. *Food Nutri Sci* 2014; 5(09): 801.

[51]. Li XM. Protective effect of *Lycium barbarum* polysaccharides on streptozotocin-induced oxidative stress in rats. *Intl J Biol Macromol* 2007; 40(5): 461-5.

[52]. Khalil OA, Ramadan KS, Danial EN, Alnahdi HS, Ayaz NO. Antidiabetic activity of *Rosmarinus officinalis* and its relationship with the antioxidant property. *Afr J Pharm Pharmacol* 2012; 6(14): 1031-6.

[53]. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Sci* 2002; 30-8.

[54]. Prabhakar PK, Doble M. Mechanism of action of natural products used in the treatment of diabetes mellitus. *Chin J Integ Med* 201; 17(8): 563-74.

[55]. Zhu JX, Wang Y, Kong LD, Yang C, Zhang X. Effects of *Biota orientalis* extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J. Ethnopharmacol* 2004; 93(1): 133-40.