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Effect of berberine in comparison to metformin on the biophysical and biochemical parameters in diabetic albino Wistar rats

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***Abstract*---**Introduction: Diabetic endothelial dysfunction is accompanied by increased oxidative stress and upregulated proinflammatory and inflammatory mediators in the endothelial vasculature. Aim of this study is to investigate the effect of Berberine, a natural alkaloid, on the oxidative stress, inflammation and its anti-oxidant effect in streptozotocin diabetic rats and to compare the effectiveness of FF with that of Metformin (Met) Material & Methods: This experimental animal study was conducted at animal house. The sample size included 174 albino wistar rats divided into 3 Groups, one control groups (C) Diabetic and untreated and two test groups. T1 Diabetic and treated with metformin 75 mg/kgwt/day) and T2 (T –

Diabetics treated with Berberine(Ber) 100 mg/kgwt/day), with 58 rats in each group (29 male & 29 female). All the rats were treated with streptozotocin intra peritoneally and the diabetic state was induced. T1 group was treated with metformin 75 mg/kg/wt/day. The T2 group of rats were treated with Berberine at a dose of 100 mg/kgwt/day. Blood sample was drawn from retro orbital plexus of animals and the biophysical and biochemical parameters were tested at an interval of 3, 6 and 12-months duration. Comparison was done between the metformin treated control group and Berberine treated test group. Results: Test of statistics, one way Analysis of Variance (ANNOVA) was used to compare the groups. Dunnet's test was used to do a multiple compression. Berberine with a dose of 100 mg/kgwt/day was significant in comparison with metformin on the biophysical (body weight), biochemical parameters (RBS (random blood sugar), urea, creatinine, HbA1C, Total cholesterol, Triglycerides, HDL -C, LDL-C, Inflammatory cytokines, TNF Alpha tissue kidney, PPAR Alpha tissue kidney, NfKB tissue kidney and on the oxidative stress (MDA) and on antioxidant status (SOD) in diabetic rats. Conclusion: Our findings suggest that Berberine, a natural alkaloid, generates a protective effect in diabetes induced rats from progression of diabetes and there in preventing the diabetic complications. This may represent a novel treatment strategy along with the existing treatment strategies to limit microvascular injury related to diabetes mellitus.

***Keywords*---**berberine (Ber), metformin (Met), streptozotocin (STZ), anti inflammatory markers, antioxidative stress markers, anti-oxidants.

Introduction

Diabetes Mellitus (DM) is a metabolic, endocrine disorder. It is a chronic non-communicable disease which generally starts insidiously (over a period of long time), and even in the absence of symptoms (hence called as a silent killer). Many individuals are accidently detected as a case of DM when they are investigated for some other reasons like preoperative investigations. It is characterized by a state of hyperglycemia (high blood sugar level) due to insulin deficiency. n the year 2012, DM was a direct cause of death of 1.5 million people and most of them (80%) belonged to low and middle income countries. Asian countries contribute to more than 60% of world's diabetic burden. The prevalence of DM is expected to raise from 285 million in 2010 to 438 million cases of diabetes in 2030. WHO projects that DM will be a 7th leading cause of death in 2030[1]. More than 80% of diabetes deaths occur in low- and middle-income countries[4].WHO projects that diabetes will be the 7th leading cause of death in 2030[5]

Diabetic patients develop vascular complications at a much faster rate in comparison to nondiabetic individuals, and cardiovascular risk is increased up to tenfold [5]. Endothelial dysfunction and oxidative stress play a key role in the pathogenesis of diabetic vascular disease [6]. DM is characterized by hyperglycemia and hyperlipidemia, two cardinal biochemical features associated

with inhibition of endothelial nitric oxide synthase (eNOS), leading to diminished NO production and increased formation of reactive oxygen species (ROS) in endothelial and vascular smooth muscle cells. Besides, impaired expression or activity of some antioxidant enzymes such as superoxide dismutase (SOD) and catalase contributes to the development of endothelial dysfunction in DM by increasing oxidative stress [7]. Endothelial dysfunction accompanied by upregulated proinflammatory and inflammatory mediators is thought to be another contributing factor to the pathogenesis of diabetic vascular complications. Multiple effects of inflammatory cytokines like interleukin-1 (IL-1) and tumor necrosis factorα (TNF-α), which lead to prothrombotic and proinflammatory changes on the vascular endothelium, have been outlined in some reports [8].The United Kingdom Prospective Diabetes Study (UKPDS) has revealed that metformin is the only oral anti-hyperglycemic agent that reduces macrovascular complications in patients with T2DM [3]. Further clinical investigations and practice have recommended it as a first line drug for T2DM.Metformin suppresses hepatic glucose production and stimulates glucose uptake in muscle and adipose, resulting in improvement of hyperglycemia and hyperlipidemia and alleviating non-alcoholic fat liver disease (NAFLD) [17–19]. In addition to enhancing insulin sensitivity in peripheral tissues, metformin can protect b-islets against lipotoxicity and glucotoxicity to restore insulin secretion [20, 21]. The first target of metformin identified is the 5′-AMP activated protein kinase (AMPK), although some effects are reported to be mediated through AMPK-independent mechanisms [22–24]

Berberine belongs to the structural class of protoberberines and is also present in the barks of other plant species including Hydrastis Canadensis L., Coptis chinensis Franch, Arcangelisia flava (L.) Meer., B. aquifolium Pursh. and B. aristata D.C. [7]A meta-analysis of 21 clinical trials revealed that berberine has therapeutic effects on T2DM, hyperlipidemia and hypertension, comparable to other therapeutic regimes [29]. Studies have indicated that, similarly to metformin, berberine executes its functions by regulating a variety of effectors including AMPK, MAPK, PKC, PPARa, PPARg [28, 30]. Tremendous amount of efforts has been casted to identify effective agents with low toxicity. Therefore, the aim of this study was to test the effectiveness of natural alkaloid berberine in comparission with metformin in exerting hypoglycemic, hypolipidemic antioxidant, anti oxiditive stress, anti-inflammatory effect in STZ induced diabetic albino wistar rats.

Material & Methods

Animals

It was an animal based experimental study conducted at Animal House of Faculty of Palamur Bioscience Private Limited for a period of one year.The study was approved by Institutional Animal Ethics Committee (IAEC) Palamur Biosciences Private Limited. CPCSEA Registration Number -1312/PO/ReBiBt-S/ 09/CPCSEA. Animals were obtained from in house bred at Palamur Biosciences Pvt. Ltd.

Experimental design

All the animals were fed by standard rat pellet diet and were allowed for free access to water. The rats were housed in standard cages at a constant temperature (15 ^{0} – 25 ^{0} Centigrade) with fixed 12: 12-hour light-dark cycle. The sample size included 116 albino wistar rats divided into 3 Groups, one control groups (C) and two test groups T1 and T2, with 58 rats in each group (29 male & 29 female). All the rats in the group were subjected to overnight fasting. Next day, all the rats were treated with streptozotocin (55 mg/kgwt) intra peritoneally before use was dissolved in 0.1 M in freshly prepared sodium citrate buffer, pH 4.5, made isotonic by the addition of 0.25M NaCl. [18-20]. 5% of glucose water was given for two days, to prevent drug-induced hypoglycaemic shock.

Seven days after the administration of STZ injection, the blood sample was collected from retroorbital plexus and centrifuged at 1000 x g for 20 minutes and the serum (supernatant) was collected. Blood glucose levels were determined by using the commercial kits by semi autoanalyzer the rats with blood glucose levels of 200mg/dl or more were considered as diabetic. Control group was monitored without any treatment. T1 was treated with metformin (75 mg/kgwt/day) by oral gavage. The test group T2 of rats were treated with Feno fibrate (FF) at a dose of 100 mg/kgwt/day by oral gavage.

Sample collection and tissue preparation

Blood sample was collected from retro orbital plexes of eye with the help of hematocrit capillaries (SD-Fine Pvt ltd).Rats were individually caged for 24 hours in metabolic cages and the urine sample was collected. Animals were sacrificed. Left kidney was removed and immediately preserved in 10% buffered formalin solution for histopathological examination.Right kidney was removed and washed with phosphate buffer and then homogenized in a homogenizing buffer (0.1 M phosphate buffer, pH 7.4) using telon homogenizer. The homogenate was centrifuged t 9000g for 20 minutes to remove debris.The supernatant was further centrifuged at 15,000g for 20 minutes and the supernatant was used for various biochemical assays. Following investigations were performed at an interval of 3 ,6 and 12 months duration. Body weight, organ weights (kidney and eye) were measured.

Random blood sugar (RBS) mg/dl, Glycosylated hemoglobin (HbA1C %), Urea mg, Creatine mg, Urine albumin, (TC)Total cholesterol, (TG) Triglycerides, High density lipoprotein (HDL-C), Low density lipoprotein (LDL-C), Tumor necrosis factor alpha (Tissue kidney TNFα), ,Peroxisome proliferator activated receptor (PPAR α tissue kidney) nuclear factor kappa light chain enhancer of activated B cells (NfKB tissue kidney), Malonaldehyde (MDA), Super oxide dismutase (SOD)

Determination of RBS and HbA1C

RBS in mg/dl was estimated by semiauto analyser using erba blood glucose kit. Method used was Trinder's method [21]. Standard procedure as per the instruction manual was followed. HbA1C % was measured by HPLC method

[22,23] in Bio-Rad D 10 instrument. Standard procedure as per the instruction manual was followed.

Evaluation of kidney function

Urea in mg was estimated by semiauto analyser using erba blood urea kit. Method used was Urease-GLDH-fixed time method [24,25]. Creatine in mg was estimated by semi autoanalyzer using erba kit. Method followed was Jaffe's method [26]. Urine protein in mg/dl was estimated by nephelometry method. Standard procedure as per the instruction manual was followed [27]

Evaluation of lipid profile

TC was estimated by CHOD-PAP method in semi autoanalyzer. This reagent is based on the formulation of Allain et al and the modification of Roeschlau with further improvements to render the reagent stable in solution [28,29]. TG was estimated by GPO-Tinder, end point method in semiauto analyser. This reagent is based on the method of Wako and the modifications by McGowan et al and Fossati et al [30,31]. HDL-C was estimated by phosphotungstic acid method in semi auto analyser [32-34]. LDL-C was calculated by using Friedewald formula [35-39]. Standard procedure as per the instruction manual was followed

Evaluation of inflammatory cytokines

Tumor necrosis factor alpha (Serum TNFα) was estimated in ELISA reader.It is a kit based on sandwitch enzyme- linked immune -sorbent assay technology[40]. NfKB serum was estimated in ELISA reader. It is a kit based on sandwitch enzyme- linked immune -sorbent assay technology [41]. PPAR α in tissue kidney homogenate was estimated in ELISA reader. It is a kit based on sandwitch enzyme- linked immune -sorbent assay technology [42,43]. TNF α in tissue kidney homogenate was estimated by ELISA reader. [44]. NfKB in tissue kidney homogenate was estimated by ELISA reader. Standard procedure as per the instruction manual was followed [45].

Evaluation of lipid peroxidation

MDA levels were measured in the serum by spectrophotometric method. Standard procedure as per the instruction manual was followed [46-48].

Evaluation of anti-oxidant status

SOD levels were estimated by spectrophotometer. Standard procedure as per the instruction manual was followed [49,50].

Statistical Analysis

Data has been entered in MS XL software. Frequency distribution tables have been calculated and tabulated. Test of statistics, one way Analysis of Variance (ANNOVA) was used to compare the groups. Dunnet's test was used to do a multiple compressions and test of significance was tested at $p<0.001$

Table 1: Comparison of the Biophysical and Biochemical parameters among diabetic untreated controls (C) and test T1 Diabetic treated controls with metformin 75 mg/kg/wt/day and T2 Diabetic group treated with Berberine with 100 mg/kgwt/day for a period of 3 months

S.no	PARAMETERS	C Untreated 3m Mean ± SE	T1 Treated 3m	T2 Berberine 100 Test 3 m
1.	Body wt(gms)	188.6±1.601	195.5±6.054	106.5±1.601
2.	RBS mg/dl	213.1±9.471	187.1±3.206	184.7±8.639
3.	HbA1C %	6.249±0.197	7.154±0.156	6.996±0.204
4.	Urea mg	35.49±1.86	36.96±1.198	36.40±1.738
5.	Creatine mg	0.904±0.080	0.801±0.025	0.804±0.078
6.	(TC)Total cholesterol mg/dl	89.50±2.33	79.14±1.606	79.01±2.294
7.	(TG) Triglycerides mg/dl	133.5±1.990	118.2±2.991	122.6±3.104
8.	HDL-C mg/dl	19.06±0.389	16.12±0.446	16.10±0.350
9.	LDL-C mg/dl	43.75±2.33	39.39±1.840	38.38±2.416
10.	PPAR Alpha tissue kidney ng/ml	2.270±45.13	2.413±0.164	2.203±45.13
11.	TNF Alpha tissue kidney pg/ml	76.01±2.330	71.86±1.522	73.00±2.438
12.	NfKB tissue kidney ng/ml	6.768±0.176	5.692±0.224	5.804±0.197
13.	MDA nmol/ml	10.63±45.71	7.106±0.182	7.051±45.92
14.	SOD U/ml	2.526±0.124	2.631±0.193	2.613±0.194

Table 2: Comparison of the Biophysical and Biochemical parameters among diabetic untreated controls (C) and test T1 Diabetic treated controls with metformin 75 mg/kg/wt/day and T2 Diabetic group treated with Berberine with 100 mg/kgwt/day for a period of 6 months

S.no	PARAMETERS	C Untreated 6 m Mean ± SE	T1 Treated 6 m	T2 Berberine 100 Test 6 m
1.	Body wt(gms)	185.6±1.0	232.6±8.58	130.6±1.601
2.	RBS g/dl	298.07±7.54	163.48±1.28	164.9±8.787
3.	HbA1C %	7.12±0.15	6.38 ±0.10	6.398±0.207
4.	Urea mg	49.55±1.50	31.14±1.05	30.74±1.768
5.	Creatine mg	1.38±0.08	0.738±0.012	0.752±0.079
6.	(TC)Total cholesterol mg/dl	125.56±1.41	66.59±1.15	69.23±2.334
7.	(TG) Triglycerides mg/dl	146.55±1.16	108.40±1.59	110.2±3.157
8.	HDL-C mg/dl	16.68±0.35	19.05±0.35	18.13±0.356
9.	LDL-C mg/dl	79.56±1.46	25.86±1.267	29.05±2.457

10.	PPAR Alpha tissue kidney ng/ml	1.80±0.03	2.70±0.133	2.364±45.90
11.	TNF Alpha tissue kidney pg.ml	107.7±2.78	69.03±1.098	71.01±2.526
12.	NfKB tissue kidney ng/ml	7.58±0.13	3.32±0.181	3.866±0.204
13.	MDA nmol/ml	12.40±0.23	6.06±0.10	6.488±47.59
14.	SOD U/ml	1.80±0.05	3.49±0.109	2.971±0.201

Table 3: Comparison of the Biophysical and Biochemical parameters among diabetic untreated controls (C) and test T1 Diabetic treated controls with metformin 75 mg/kg/wt/day and T2 Diabetic group treated with Berberine with 100 mg/kgwt/day for a period of 12 months

S.no	PARAMETERS	C Untreated 12 m Mean ± SE	T1 Treated 12 m	T2 Berberine 100 Test 12 m
1.	Body wt(gms)	172.32±1.13	267.64±4.049253	152.6±1.371
2.	RBS g/dl	386.03±7.56	136.62±1.356256	138.3±7.525
3.	HbA1C %	8.64±0.095	5.36±0.070969	5.781±0.178
4.	Urea mg	67.47±1.15	21.40±0.472228	23.40±1.514
5.	Creatine mg	1.80±0.065	0.60±0.016502	0.662±0.068
6.	(TC)Total cholesterol mg/dl	143.29±1.48	58.41±0.77563	62.85±1.998
7.	(TG) Triglycerides mg/dl	155.23±1.19	95.197±1.358433	102.3±2.703
8.	HDL-C mg/dl	15.15±0.135	21.105±0.291852	19.62±0.305
9.	LDL-C mg/dl	97.09±1.376	18.27±0.84227	22.77±2.104
10.	PPAR Alpha tissue kidney ng/ml	74.11±46.112	3.96±0.066577642	3.612±39.31
11.	TNF Alpha tissue kidney pg/mk	122.17±1.502	59.20±0.807594282	62.86±1.444
12.	NfKB tissue kidney ng/ml	8.46±0.096	2.38±0.103634064	2.622±0.171
13.	MDA nmol/ml	14.48±0.275	3.69±0.096724	3.651±40.00

14.	SOD U/ml	1.17±0.027	4.47±0.10	3.912±0.169

Results: Table1, 2 and 3

Body weight

When compared with the (C) untreated diabetic rats (188.6±1.601), there was increase in the bodyweight within the group treated with metformin (T1) (195.5±6.054) and significant decrease in weight in the group treated with Berberine(T2) (106.5±1.601) ($p<0.0001$) for a period of 3 months. In 6 months, period, when compared with the C group (185.6±1.0), there was significant increase in the body weight in T1 group (232.6±8.58) and significant decrease in T 2 group (130.6±1.601). During a period of 12 months, when compared with group C (172.32±1.13), the body weight in group T1 (267.64±4.049253) increased significantly and T2 decreased significantly (152.6±1.371)

RBS

When compared with the (C) untreated diabetic rats (213.1±9.471), there was significant decrease in the RBS within the group treated with metformin T1(187.1±3.206) and significant decrease in RBS in the group treated with Berberine (T2) (184.7±8.639) ($p<0.0001$) for a period of 3 months. In 6 months, period, when compared with the C group (298.07±7.54), there was significant decrease in the RBS in T1 group (163.48±1.28) and significant decrease in T 2 group (164.9±8.787). During a period of 12 months, when compared with group C (386.03±7.56), the RBS in group T1 (136.62±1.356256) and T2 decreased significantly (138.3±7.525).

HbA1C

When compared with the (C) untreated diabetic rats (6.249±0.197), there was slight increase in the HbA1C within the group treated with metformin T1(7.154±0.156) and slight increase in HbA1C in the group treated with Berberine T2 (6.996±0.204) for a period of 3 months. In 6 months, period, when compared with the C group (7.12±0.15), there was slight decrease in the HbA1C in T1 group (6.38 ±0.10) and slight decrease in T 2 group (6.398±0.207). During a period of 12 months, when compared with group C (8.64±0.095), the HbA1C in group T1 (5.36±0.070969) and T2 (5.781±0.178) is decreased significantly.

Kidney function parameters

Urea

When compared with the (C) untreated diabetic rats (35.49±1.86), there was slight increase in the urea within the group treated with metformin T1(36.96±1.198) and slight increase in urea in the group treated with Berberine T2 (36.40±1.738) for a period of 3 months. In 6 months, period, when compared with the C group (49.55±1.50), there was slight decrease in the urea in T1 group (31.14±1.05) and slight decrease in T 2 group (30.74±1.768). During a period of 12 months, when

compared with group C (67.47±1.15), the urea in group T1 (21.40±0.472228) and T2 (23.40±1.514) is decreased significantly.

Creatinine

When compared with the (C) untreated diabetic rats (0.904±0.080), there was slight decrease in the creatinine within the group treated with metformin T1(0.801±0.025) and slight decrease in creatinine in the group treated with Berberine T2 (0.804±0.078) for a period of 3 months. In 6 months, period, when compared with the C group (1.38±0.08), there was slight decrease in the creatinine in T1 group (0.738±0.012) and slight decrease in T 2 group (0.752±0.079). During a period of 12 months, when compared with group C (1.80±0.065), the creatinine in group T1 (0.60±0.016502) and T2 (0.662±0.068) is decreased.

Lipid profile

Total Cholesterol

When compared with the (C) untreated diabetic rats (89.50±2.33), there was slight decrease in the TC within the group treated with metformin T1(79.14±1.606) and slight decrease in TC in the group treated with Berberine T2 (79.01±2.294) for a period of 3 months. In 6 months, period, when compared with the C group (125.56±1.41), there was slight decrease in the TC in T1 group (66.59±1.15) and slight decrease in T 2 group (69.23±2.334). During a period of 12 months, when compared with group C (143.29±1.48), the TC in group T1 (58.41±0.77563) and T2 (62.85±1.998) is significantly decreased.

HDL-C

When compared with the (C) untreated diabetic rats (19.06±0.389), there was slight decrease in the HDL-C within the group treated with metformin T1(16.12±0.446) and slight decrease in HDL-C in the group treated with Berberine T2 (16.10±0.350) for a period of 3 months. In 6 months, period, when compared with the C group (16.68±0.35), there was slight increase in the HDL-C in T1 group (19.05±0.35) and slight increase in T 2 group (18.13±0.356). During a period of 12 months, when compared with group C (15.15±0.135), the TC in group T1 (21.105±0.291852) and T2 (19.62±0.305) is significantly increased.

LDL-C

When compared with the (C) untreated diabetic rats (43.75±2.33), there was slight decrease in the LDL-C within the group treated with metformin T1(39.39±1.840) and slight decrease in LDL-C in the group treated with Berberine T2 (38.38±2.416) for a period of 3 months. In 6 months, period, when compared with the C group (79.56±1.46), there was significant decrease in the LDL-C in T1 group (25.86±1.267) and significant decrease in T 2 group (29.05±2.457). During a period of 12 months, when compared with group C (97.09±1.376), the LDL-C in group T1 (18.27±0.84227) and T2 (22.77±2.104) is significantly decreased.

Triglycerides

When compared with the (C) untreated diabetic rats (133.5±1.990), there was slight decrease in the LDL-C within the group treated with metformin T1(118.2±2.991) and slight decrease in LDL-C in the group treated with Berberine T2 (122.6±3.104) for a period of 3 months. In 6 months, period, when compared with the C group (146.55±1.16), there was significant decrease in the LDL-C in T1 group (108.40±1.59) and significant decrease in T 2 group (110.2±3.157). During a period of 12 months, when compared with group C (155.23±1.19), there was significant decrease in the LDL-C in T1 group (95.197±1.358433) and significant decrease in T 2 group (102.3±2.703).

Inflammatory cytokine

TNF α tissue homogenate(kidney)

When compared with the (C) untreated diabetic rats (76.01±2.330), there was slight decrease in the TNF α within the group treated with metformin T1(71.86±1.522) and slight decrease in TNF α in the group treated with Berberine T2 (73.00±2.438) for a period of 3 months. In 6 months, period, when compared with the C group (107.7±2.78), there was slight decrease in the TNF α in T1 group (69.03±1.098) and slight decrease in T 2 group (71.01±2.526). During a period of 12 months, when compared with group C (122.17±1.502), the TNF α in group T1 (59.20±0.807) and T2 (62.86±1.444) is significantly decreased.

NfKB tissue homogenate (kidney)

When compared with the (C) untreated diabetic rats (6.768±0.176), there was slight decrease in the NfKB within the group treated with metformin T1(5.692±0.224) and slight decrease in NfKB in the group treated with Berberine T2 (5.804±0.197) for a period of 3 months. In 6 months, period, when compared with the C group (7.58±0.13), there was significant decrease in the NfKB in T1 group (3.32±0.181) and significant decrease in T 2 group (3.866±0.204). During a period of 12 months, when compared with group C (8.46±0.096), the NfKB in group T1 (2.38±0.103) and T2 (2.622±0.171) is significantly decreased.

PPAR Alpha tissue homogenate (kidney)

When compared with the (C) untreated diabetic rats (2.270±45.13), there was slight increase in the NfKB within the group treated with metformin T1(2.413±0.164) and slight increase in NfKB in the group treated with Berberine T2 (2.203±45.13) for a period of 3 months. In 6 months, period, when compared with the C group (1.80±0.03), there was significant increase in the NfKB in T1 group (2.70±0.133) and significant increase in T 2 group (2.364±45.90). During a period of 12 months, when compared with group C (74.11±46.112), the NfKB in group T1 (3.96±0.066) and T2 (3.612±39.31) is significantly increased.

Oxidative stress

MDA

When compared with the (C) untreated diabetic rats (10.63±45.71), there was significantly decreased in the MDA within the group treated with metformin T1(7.106±0.182) and significantly decreased in MDA in the group treated with Berberine T2 (7.051±45.92) for a period of 3 months. In 6 months, period, when compared with the C group (12.40±0.23), there was significant decrease in the MDA in T1 group (6.06±0.10) and significant decrease in T 2 group (6.488±47.59). During a period of 12 months, when compared with group C (14.48±0.275), the MDA in group T1(3.69±0.096) and T2 (3.651±40.00) is significantly decreased.

SOD

When compared with the (C) untreated diabetic rats (2.526±0.124), there was slight increase in the SOD within the group treated with metformin T1(2.631±0.193) and slight increase in SOD in the group treated with Berberine T2 (2.613±0.194) for a period of 3 months. In 6 months, period, when compared with the C group (1.80±0.05), there was significant increase in the SOD in T1 group (3.49±0.109) and significant increase in T 2 group (2.971±0.201). During a period of 12 months, when compared with group C (1.17±0.027), the SOD in group T1 (4.47±0.10) and T2 (3.912±0.169) is significantly increased.

Discussion

Our study demonstrated that Berberine in comparison with metformin is effective in controlling the glycaemic levels, preserving the kidney function, normalizing the lipid profile, decreasing the inflammatory cytokines, decreasing the oxidative stress and increasing the anti-oxidant status. In our study over a period of one year, there was an increase in the weight, in group treated with metformin and decrease in weight significantly in the group treated with Berberine. This can be justified by the anti-obese activity is consistent with the finding that berberine significantly decreased sizes and number of lipid droplets in 3T3-L1 adipocytes [51]. Berberine exerts its long-term body weight losing effect through enhancing AMPK-mediated ATGL expression, which increases the basal lipolysis state of triglycerides in adipocytes [51]. Our findings were similar with Zhang Z etal ‘s study in 2014, where he found mice treated with berberine were found to contain shrunk adipocytes [52]. We found that the random blood sugar and HbA1C was significantly reduced in the groups treated with metformin and berberine, when compared with the untreated control group. Metformin suppresses hepatic glucose production and stimulates glucose uptake in muscle and adipose, resulting in improvement of hyperglycemia and hyperlipidemia and alleviating non-alcoholic fat liver disease (NAFLD) [53-55]. In addition to enhancing insulin sensitivity in peripheral tissues, metformin can protect b-islets against lipotoxicity and glucotoxicity to restore insulin secretion [56,57]. The first target of metformin identified is the 5′-AMP activated protein kinase (AMPK), although some effects are reported to be mediated through AMPK-independent mechanisms [58-60]. Yin et al compared the effects of berberine and metformin [61]. In a three months trial, 36 patients with T2DM were randomly assigned with berberine or

metformin. It was found that hypoglycemic effect of berberine is comparable to that of metformin. The level of hemoglobin A1c (HbA1c), fasting and postprandial glucose decreased by 7.5%, 6.9% and 11.1% respectively at the end of the trial. Similar findings were reported in a clinical study of Zhang et al [28]. To be noteworthy, via activation of AMPK, berberine can stimulate glucose uptake in muscle, liver and adipose, and inhibit gluconeogenesis in liver by downregulation of gluconeogenic enzymes (phosphoenolpyruvate carboxyl kinase and glucose-6-phosphatase) [62]. We noticed the concentration of blood urea and serum creatinine was significantly decreased in the groups treated with metformin and berberine when compared with the untreated controlled group. These findings were similar to the studies by Liu et al., 2008[63] and Wu et al., 2012 [64]. There was a significant improvement in the lipid profile, decrease in TC, LDL-C, Triglycerides and increase in HDL-C levels in the groups treated with metformin and berberine. Berberine was reported to inhibit cholesterol and triglyceride synthesis in HepG2 cells, a human hepatoma cell line, and primary hepatocytes [65,66]. The evidence for the inhibitory effect of berberine on NAFLD comes from rodent animal models that are induced by fat diet [67].Our study over a period of one year, reported decreased levels of TNF α,NFKB and increased levels of PPARα levels in the groups treated with berberine and metformin. NF-κβ plays key role in pathogenesis of vascular complications of diabetes. Persistent hyperglycemia activates NF-κβ that triggers expression of various cytokines, chemokines and cell adhesion molecules. Over-expression of TNF-α, interleukins, and other pro-inflammatory proteins and pro-apoptotic genes by NF-κβ is key risk factor in vascular dysfunction. Inhibition of NF-κβ pro-inflammatory pathway is upcoming novel target for management of vascular complications of diabetes. Similar effects were observed with metformin. Study of Koh et al indicates that metformin inhibits NFkB activation in intestinal epithelial cells [68]

SOD is an important defense enzyme which neutralizes the effect of superoxide anion during the oxidative stress in the tissues. Oxidative stress generally causes damage to the membrane polyunsaturated fatty acids (PUFA) leading to generation of malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS). Several studies have indicated an increase in serum TBARS and a decrease in plasma SOD activity signifying an imbalance between the prooxidant and antioxidant states in the body, leading to an imbalance in systemic redox status [69], There was decrease in the MDA levels in groups treated with metformin and FF, signifying the decrease in oxidative stress. There was increase in the antioxidant status (SOD) in the group treated with metformin and Berberine.

Limitations

Due to the ethical issues, the diabetes free rats were not sacrificed and did not consider as controls. Studies with combination of fenofibrate, metformin and natural alkaloids are anticipated as these will reduce the cost burden on the society in the treatment of diabetic patients and its related micro and macrovascular complications.

Conclusion

In our study we demonstrated that Berberine in comparison with metformin, at low doses, generates protective action and prevent the diabetic micro vascular complications in relation to diabetes in rats with STZ induced diabetes. These findings may represent a novel treatment strategy to limit complications in patients with type 2 diabetes mellitus. It is postulated that the similar effects of berberine and metformin is attributed to similar anti-diabetic mechanisms in spite of different transporter and metabolism. Therefore, combination of these two drugs might allow reduction in dosage of each individual drugs to solve problems such as oral bioavailability of berberine and side effects of each alone. Since the side effects of berberine are tolerable and controllable (usually gastrointestinal discomfort), clinical investigations to compare these two drugs are definitely feasible. Hopefully, more clinical trails on the use of berberine will be conducted in the near future.

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Conflict of interest – No conflict of interest declared

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