Original Research Article Medicinal Chemistry

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIDIABETIC PROPERTIES OF A NEW METFORMIN-3- HYDROXYFLAVONE COMPLEX STUDIED IN HIGH FAT DIET FED - LOW DOSE STREPTOZOTOCIN INDUCED EXPERIMENTAL TYPE 2 DIABETES IN WISTAR RATS

C. JAIGANESH[1], V. ROSHANA DEVI[1], S. IYYAM PILLAI[2] AND S. SUBRAMANIAN[1]*

[1]Department of Biochemistry, University of Madras, Guindy Campus, Chennai-25
[2]PG and Research Department of Chemistry, Pachaiyappa's College, Chennai-30

ABSTRACT

Diabetes mellitus is a multifactorial, multisystemic chronic metabolic endocrine disorder arises due to absolute (T1DM) or insufficient secretion of insulin coupled with insulin resistance (T2DM). T2DM accounts for more than 90% of the diabetic population and its incidence is increasing alarmingly worldwide. Among the currently available drugs for the treatment of diabetes such as insulin, thiazolidinediones, gliclazide analogs, α-glucosidase inhibitors, glucagon like peptide-1 receptor agonists and dipeptidyl peptidase-4-inhibitors, metformin occupies a unique importance in its clinical field for the treatment of both T1DM and T2DM due to its safety and efficacy. However, metformin causes lactic acidosis in addition to gastrointestinal tract complications relatively at high doses. Hence, several researchers attempt to synthesize various metformin complexes to minimize the dosage of metformin. The present study is aimed to synthesize a new complex using equimolar concentrations of metformin and 3-hydroxyflavone capable of eliciting maximum antidiabetic properties at a relatively less concentration. Metformin-3-hydroxyflavone complex was synthesized, characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectral studies and its safety and efficacy were evaluated in high fat diet fed-low dose STZ induced experimental type 2 diabetes in Wistar rats. The data obtained evidenced that the oral administration of the complex at a concentration of 20mg/kg.b.w./rat/day for 30 days to diabetic rats showed significant antidiabetic and antioxidant properties which was comparable to metformin treatment at a concentration of 50mg/kg.b.w.

KEYWORDS: ***T2DM, metformin, 3-hydroxyflavone, spectral studies, antidiabetic properties, oxidative stress, antioxidants***

S. SUBRAMANIAN*
Department of Biochemistry, University of Madras, Guindy Campus, Chennai-25

Received on: 06-04-2017
Revised and Accepted on : 05-05-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.3.p1-15

INTRODUCTION

Diabetes mellitus (DM) is a progressive metabolic disorder arises due to an absolute lack of insulin secretion (T1DM) or its action coupled with insufficient levels of insulin (T2DM)[1]. It is characterized by persistently elevated levels of both fasting and postprandial blood glucose levels[2]. T2DM accounts for more than 90% of the diabetic population and its prevalence is increasing alarmingly worldwide due to a sedentary lifestyle, genetic and environmental factors[3]. Several antidiabetic drugs such as insulin, metformin, thiazolidinediones, gliclazide analogs, α-glucosidase inhibitors, glucagon like peptide-1 receptor agonists and dipeptidyl peptidase-4-inhibitors with extremely different mechanisms of action are currently available for the treatment of diabetes mellitus, none is found to be ideal due to undesirable side effects in addition to diminution of action after prolonged treatment[4]. Hence, search for novel drugs with maximum efficacy and safety still continues. Metformin, a biguanide derivative is considered as the first line of treatment to T2DM and backbone for combination therapy[5]. Ever since metformin was developed as an antihyperglycemic agent by Sterne in the 1950s, it is being extensively prescribed by the clinicians for the treatment of both T1DM and T2DM[6]. On the basis of experimental and clinical evidences, the guidelines of ADA (American Diabetes Association) and EASD (European Association of the study of Diabetes) recommended metformin as a drug of the first choice in patients diagnosed with T2DM[7, 8]. Metformin is relatively well tolerated and the hypoglycemic episodes do not occur in therapeutic dosing as it has no effect on the insulin secreting pancreatic β-cells[9, 10]. Treatment with metformin primarily reduce the insulin resistance without causing weight gains which in turn more likely due to alterations in appetite or intake[11]. The glucoregulatory properties of metformin are mainly attributed to reducing hepatic glucose production and augmented insulin-mediated utilization of glucose in peripheral tissues[12]. Thus, the antihyperglycemic effect of metformin is demonstrable only in diabetic patients that too in the presence of insulin[13]. With regard to pharmacokinetics and metabolism, metformin does not appear to be metabolized in humans. Intestinal absorption reaches the peak level between 1 and 3 hours after an oral dose and the maximum blood concentration at a steady state is 4μg/ml. Its bioavailability at a therapeutic dose reaches 50%-60% [14] but may drop as the dose is increased due to the decrease in the absorption[15]. Metformin is excreted by the kidneys and the clearance rate is about four times the creatine clearance with normal renal function[16, 17]. Metformin is also excreted by the salivary glands at a markedly lower concentration when compared to plasma with a half-life of about nine hours. The unpleasant taste that the diabetic patients experience is attributed to the presence of metformin in their saliva[14]. In spite of the above credentials, metformin treatment at relatively high doses was associated with metabolic acidosis characterized by increased serum lactate and beta- hydroxybutyric acid levels and decreased serum bicarbonate and urine pH[18, 19]. Additionally, gastrointestinal tract complications are reported in at least in 10% of the diabetic patients treated with metformin[20]. A reduction in vitamin B12 absorption can also occur after a long period of metformin treatment[21]. Hence, metformin treatment should be avoided in diabetic patients whose renal functions is impaired and in those patients in whom oxygenation, tissues perfusion or liver functions are severely compromised[22]. Though metformin is widely used as monotherapy in the initial stages, a combination of one or more complex strategies in addition to metformin are included if the glycemic control targets are not achieved and finally, more complex strategies involving insulin dose escalation is certainly warranted[23]. Despite a number of combinations of metformin is currently available, none is found satisfactory due to hypoglycemic episodes and/or undesirable side effects.Experimental and clinical studies have clearly established the role of chronic hyperglycemia-induced oxidative stress in the etiology of diabetes and its secondary complications[24, 25]. Phytochemicals are ecologically derived plant secondary metabolites which protect them against environmental stress such as UV radiation, pollution, high temperature, extreme cold, drought, flood, tissue damage and microbial attacks[26]. Interestingly, these secondary metabolites are known to play a pivotal role in alleviating the primary and secondary complications of dreadful human diseases such as cancer, diabetes and atherosclerosis[27]. Among the various phytoingredients, flavonoids are known for their wide range of pharmacological as well as beneficial effects on the maintenance of human health care especially in quenching oxidative stress, a major contributory factor for the progression of secondary complications[28]. Amongst flavonoids, flavones are known to chelate the metal ions with great affinity owing to the presence of α-hydroxycarbonyl group and their ability to quench the free radicals[29]. The synthetic 3-hydroxyflavones have been found to have significant antidiabetic properties by pleoitropic and multimodal suppression of insulin resistance and enhancement of glucose uptake by skeletal muscles[30]. In view of the reports available in the literature, in the present study, an attempt has been made to synthesize a new metformin-3-hydroxyflavone complex and evaluate its antidiabetic properties in high fat diet fed-low dose STZ induced experimental type 2 diabetes in rats.

MATERIALS AND METHODS

Chemical

Metformin hydrochloride, 3-Hydroxyflavone and STZ were procured from Sigma-Aldrich, St. Louis, USA. Ultra-sensitive ELISA kit for rat insulin and C-peptide was purchased from Crystal Chem Inc. Life Technologies, India. All the other reagents used in the present study were of analytical grade.

Analytical instruments

IR spectral studies were carried out in the solid state as pressed KBr pellets using a Perkin-Elmer FT-IR spectrophotometer in the range of 400–4000 cm^{-1}. The mass spectrum of the complex was obtained using Jeol Gcmate. The ^{1}H NMR and ^{13}C NMR data were obtained at 300MHz and 500MHz, respectively, using a BrukerAM-500 instrument. The spectral analysis data

were recorded without any modification for instrumental characteristics.

Synthesis of metformin-3-hydroxyflavone complex

3-Hydroxyflavone (0.005 mol, 1.2 g) is dissolved in 25 ml of methanol and stirred in hot condition till the formation of a clear solution. To this warm solution, metformin hydrochloride (0.005 mol, 0.6458 g) dissolved in methanol (25 ml) was added in a drop wise manner. The mixture is refluxed with continuous stirring over a water bath for six hours. The resultant coloured product (L) was washed with water and methanol, dried under vacuum and recrystallized from methanol.

Experimental animals

Male Albino Wistar rats weighing around 160 to 180gm were procured from the Tamil Nadu Veterinary and Animal Sciences University, Chennai, and were housed under standard husbandry conditions (12±1 h light and dark cycle, relative humidity 55% ± 10%). The animals were fed with a balanced diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The rat pellet diet is composed of 55% nitrogen-free extract, 21% protein, 5% fat and 4% fiber (w/w) with sufficient levels of vitamins and minerals. The experimental design was conducted according to the ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines, for the examination of experimental pain in conscious animals (IAEC NO: 03/10/12).

Induction of experimental diabetes

The rats were allocated into two dietary regimens by feeding either normal pellet diet (NPD) or high fat diet (HFD) for 2 weeks of dietary manipulation. HFD contains powdered NPD-365 g/kg, lard-310 g/kg, casein-250 g/kg, cholesterol-10 g/kg, vitamin and mineral mix-60 g/kg, DL-methionine-3 g/kg, yeast powder-1 g/kg, and NaCl-1 g/kg. After 2 weeks of dietary manipulation to induce insulin resistance, Group 2, Group 3, group 4 rats were injected with a single low dose of STZ (35mg/kg b.w./rat); control rats (Group 1) fed with NPD were injected intraperitoneally with the same volume freshly prepared cold citrate buffer (pH-4.5, 0.1mol/L) [31]. On the third day after STZ injection, blood was collected from the tail vein and the fasting blood glucose levels were determined. Rats having the fasting blood glucose levels ≥ 250 mg/dl were considered as diabetic rats and chosen for further studies.

Acute toxicity and dosage fixation studies

Acute toxicity studies were performed in normal rats. Graded doses (10, 25, 50 and 100 mg/kg b.w./rat as per OECD guidelines (423) for testing of chemicals) of metformin-3 hydroxyflavone complex in aqueous suspension was administered orally. The rats were observed for four weeks following administration. The change in food consumption, fluid intake, psychomotor activities, body weight gain, changes in skin, fur, eyes, salivation, diarrhea and lethargy were continuously monitored. Macroscopic examinations were also performed on vital organs. Similarly, the dosage fixation studies were carried out by administering graded doses of metformin-3-hydroxyflavone complex (5, 10, 20 and 50 mg/kg b.w./rat/day) for 30 days to determine the dose-dependent hypoglycemic effect in high fat diet fed-low dose STZ induced diabetic rats by monitoring the fasting blood glucose levels periodically.

Experimental protocol

The animals were divided into four groups each comprising of a minimum of six animals as follows:

Group 1: Control rats.
Group 2: HFD-STZ (35mg/kg b.w./rat) induced diabetic rats
Group 3: HFD-STZ induced diabetic rats treated with metformin-3-hydroxyflavone complex (20 mg/kg.b.w./rat) for a period of 30 days.
Group 4: HFD-low dose STZ induced diabetic rats treated with metformin (50 mg/kg.b.w./rat) for a period of 30 days.

Oral glucose tolerance test

On the day prior to sacrifice, oral glucose tolerance test (OGTT) was performed in all the groups of rats. Blood samples were collected from the lateral tail vein of rats deprived of food overnight. Successive blood samples were collected at 0, 30, 60, 90, and 120 minutes following the oral administration of 2mg/kg b.w. of glucose solution[32].

HOMA-IR

As the insulin abnormality cannot be accurately detected by a single determination of insulin or glucose levels, the insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR)[33]. Insulin resistance/sensitivity was assessed by QUICKI[34].

$$\text{HOMA-IR} = \frac{\text{Fasting insulin level (}\mu\text{U/ml)} \times \text{Fasting blood glucose level (mg/dl)}}{405}$$

$$\text{QUICKI} = 1 / (\log(\text{fasting insulin } \mu\text{U/mL}) + \log(\text{fasting glucose mg/dl}))$$

Biochemical analysis

At the end of 30 days of treatment, the rats were fasted overnight, anesthetized with Ketamine (80 mg/kg b.w./rat, i.p.) and sacrificed by cervical decapitation. Blood was collected with and without anticoagulant for the separation of plasma and serum respectively. The levels of fasting blood glucose, [35] hemoglobin, [36] and glycosylated hemoglobin [37] were estimated. Insulin and C-peptide levels were estimated using Ultrasensitive ELISA kits. Plasma protein, [38] blood urea, [39] serum creatinine [40] and serum uric acid [41] levels were estimated. The activities of AST, ALT [42] and ALP [43] in

serum were assayed. Urine sugar was detected using urine strips.

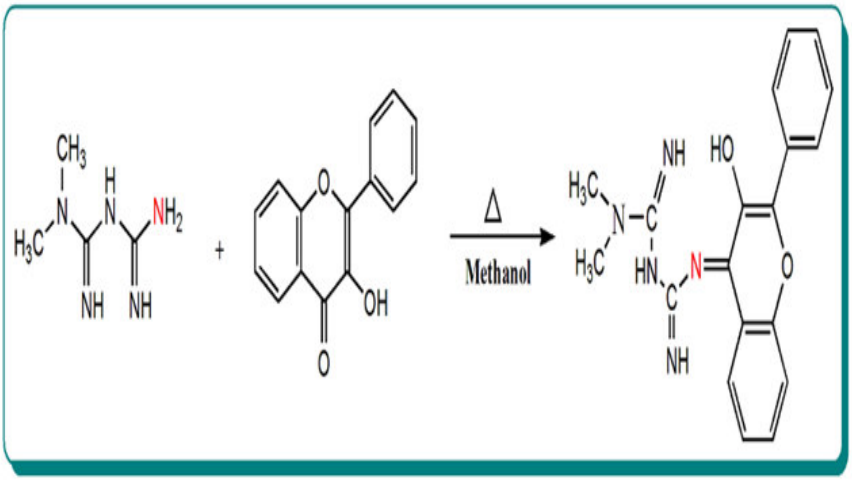
Studies on Antioxidants

The levels of lipid peroxides such as TBARS and hydroperoxides levels in the plasma and pancreatic tissues were estimated[44, 45.] The levels of non-enzymatic antioxidants such as vitamin C, vitamin E and ceruloplasmin were measured in the plasma of control and experimental groups of rats[46-48.] Similarly, the activities of enzymatic antioxidants such as SOD, catalase, GPx and GST were also assayed in the pancreatic tissues of control and experimental groups of rats[49-52.]

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM of six rats per group and the statistical significance was evaluated by "one-way analysis of variance" (ANOVA) using the SPSS (version 16) program followed by least significance test. A value of $*P < 0.05$ was considered to indicate statistical significance.

RESULTS



Scheme 1
Structure of metformin-3-hydroxyflavone

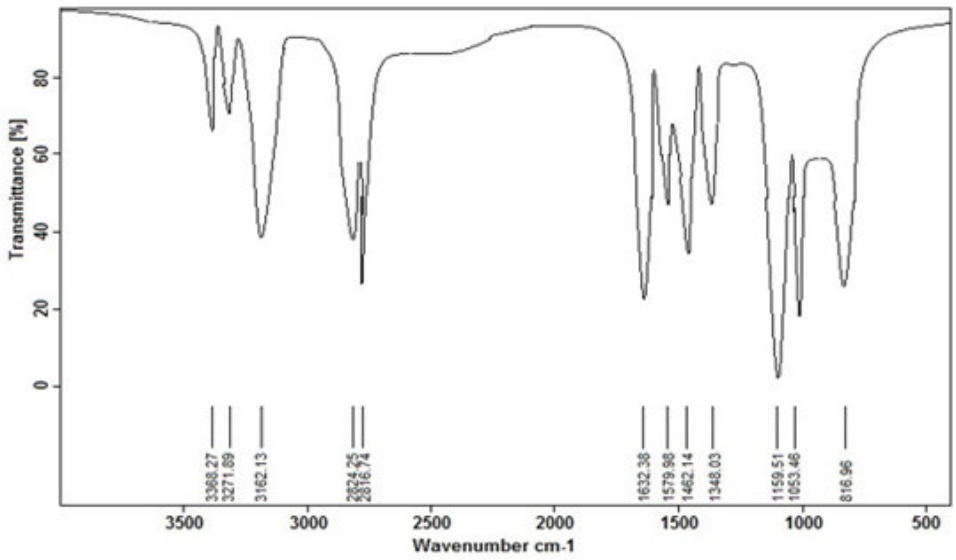


Figure 1
The IR spectrum of metformin

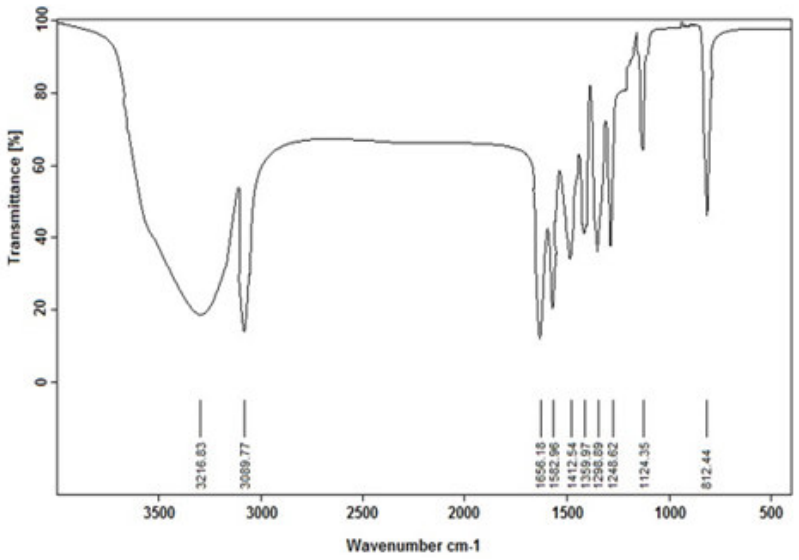


Figure 2
The IR spectrum of 3-hydroxyflavone

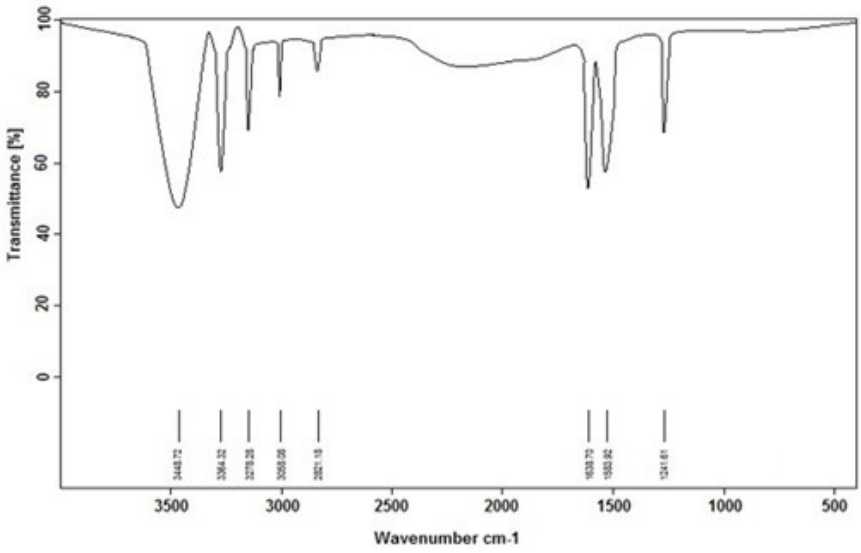


Figure 3
The IR spectrum of metformin-3-hydroxyflavone

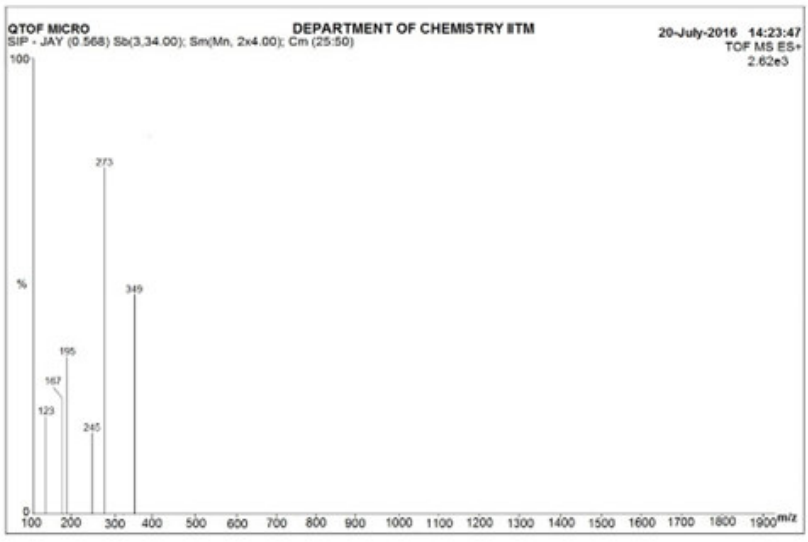


Figure 4
The mass spectrum of metformin-3-hydroxyflavone

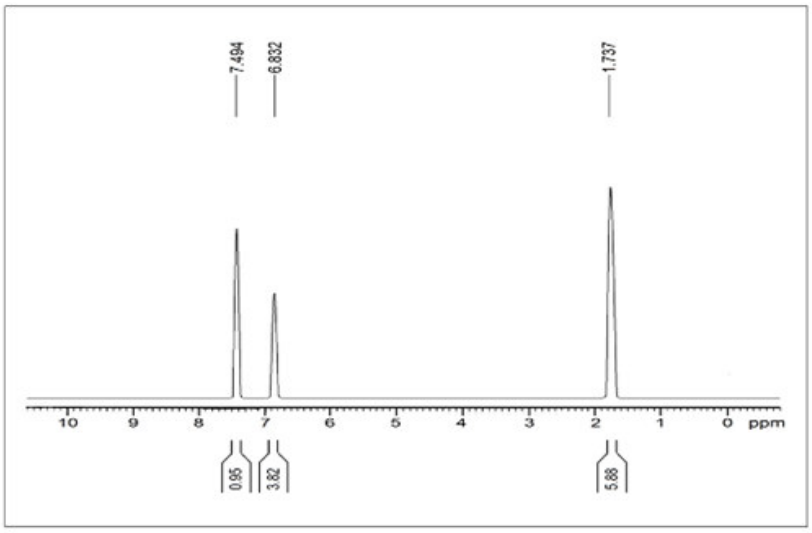


Figure 5
The ^{1}H NMR of metformin

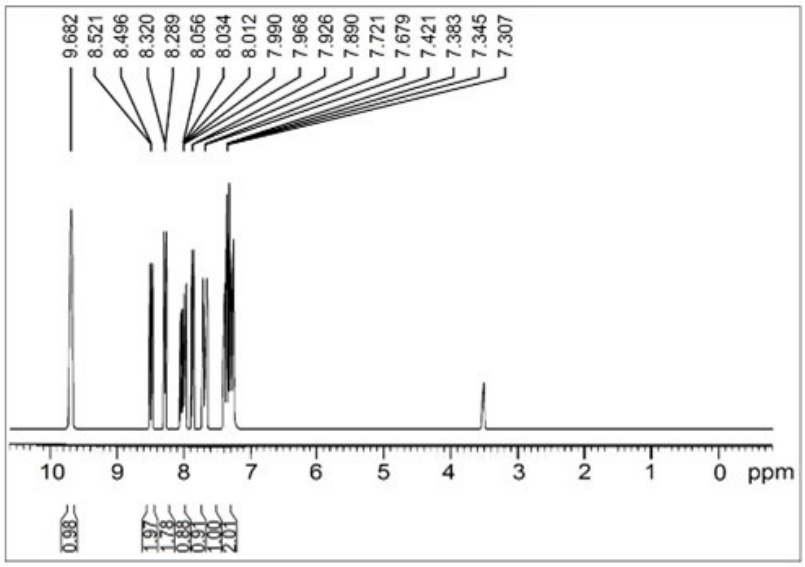


Figure 6
The ^{1}H NMR of 3-hydroxyflavone

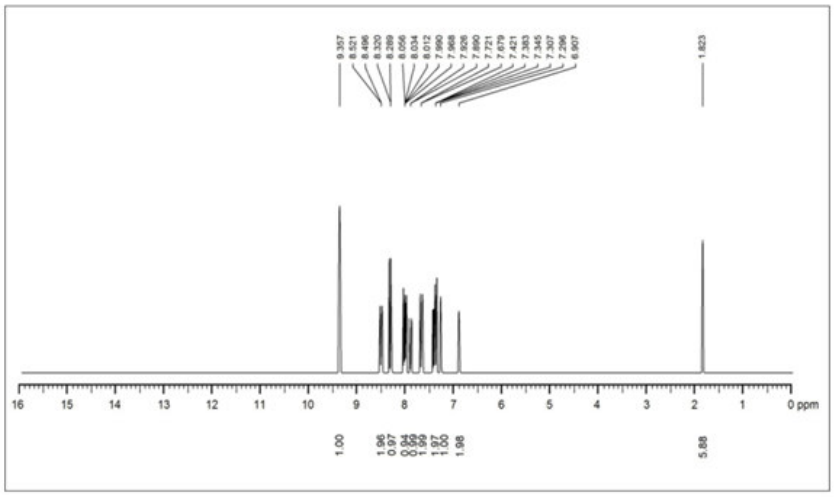


Figure 7
The ^{1}H NMR of metformin-3-hydroxyflavone

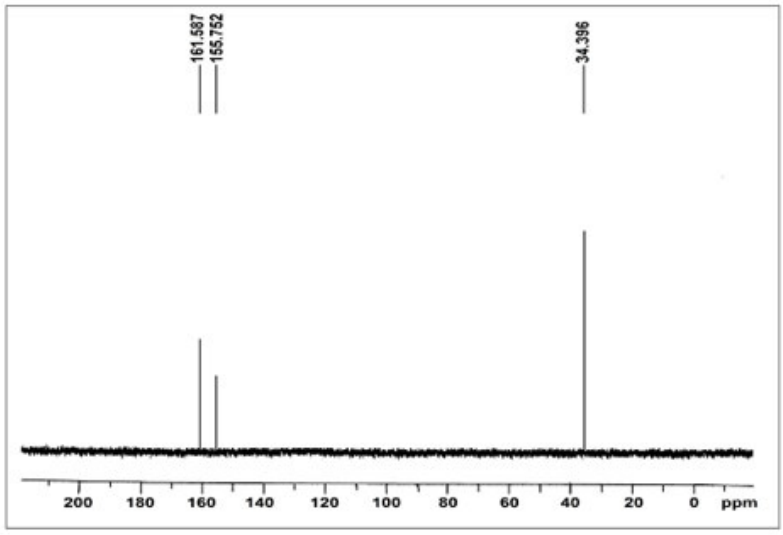


Figure 8
The ^{13}C NMR of metformin

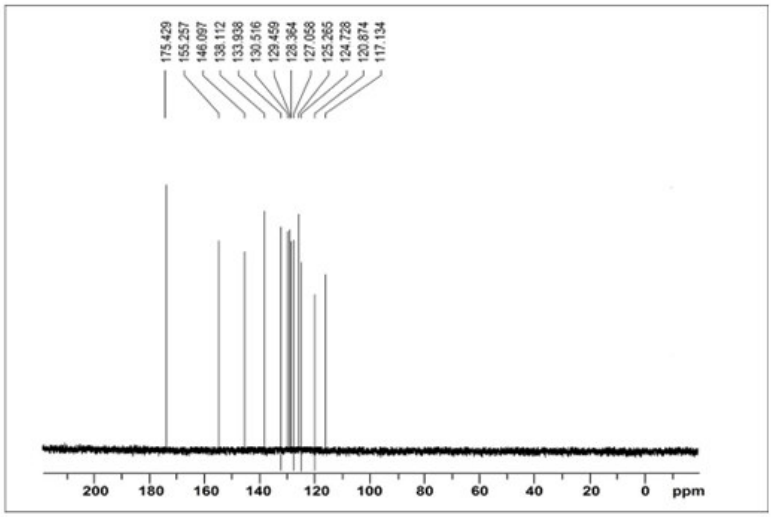


Figure 9
The ^{13}C NMR of 3-hydroxyflavone

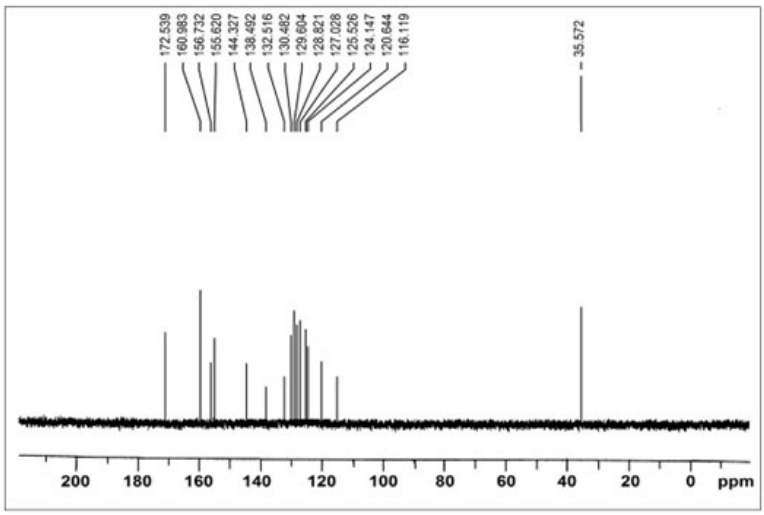
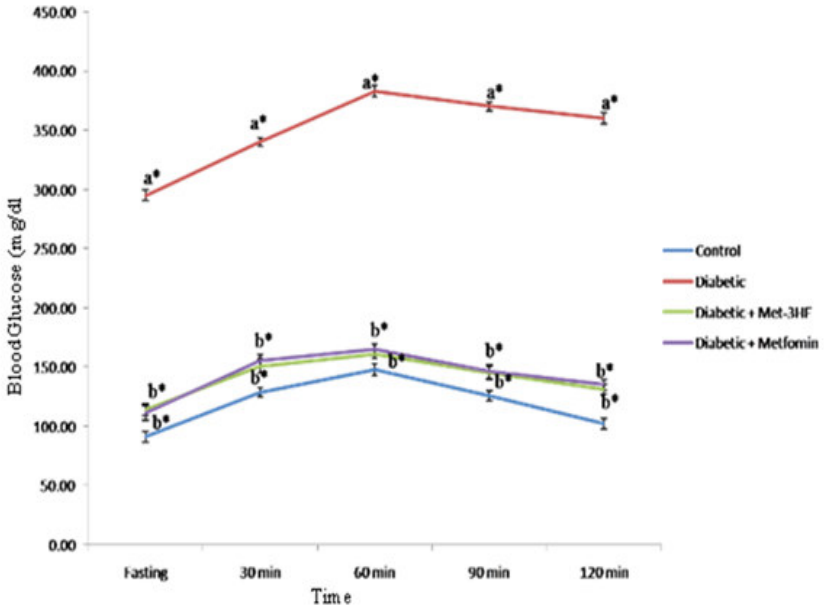
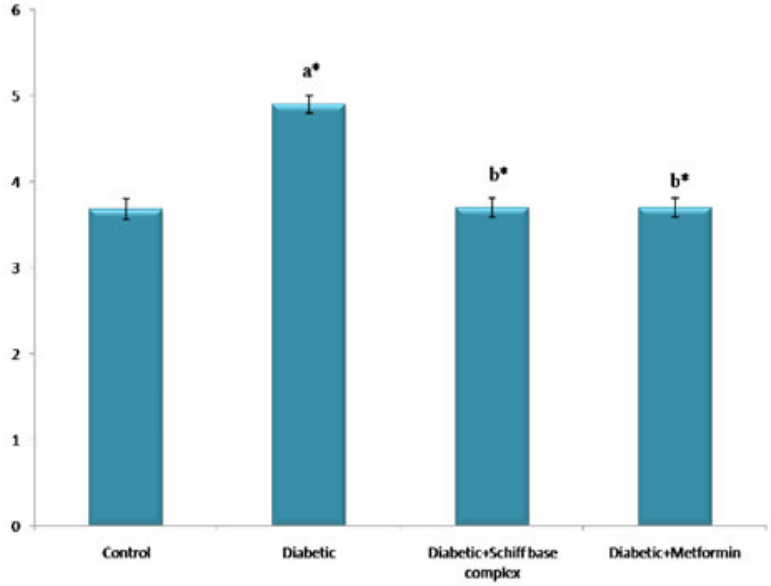


Figure 10
The ^{13}C NMR of metformin-3-hydroxyflavone complex



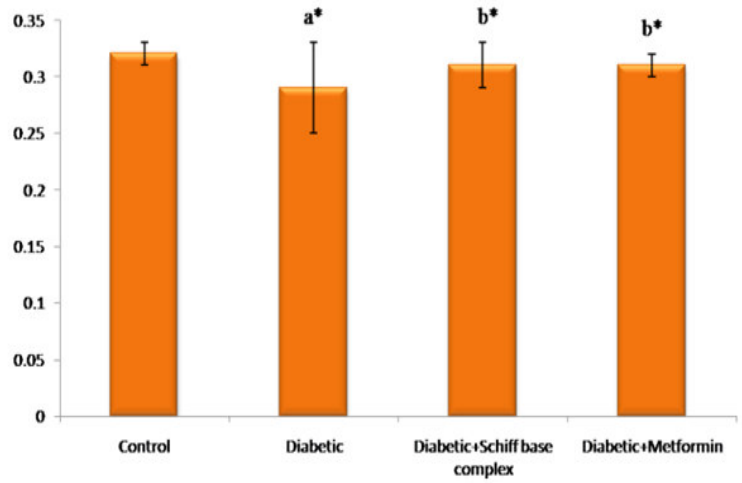
Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at $^{*}p<0.05$. The results were ^{a}compared to control rats and ^{b}compared to diabetic rats.

Figure 11
Effect of metformin-3-hydroxyflavone complex on oral glucose tolerance in the experimental groups of rats



Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at $^{*}p<0.05$. The results were ^{a}compared to control rats and ^{b}compared to diabetic rats.

Figure 12
Effect of metformin-3-hydroxyflavone complex on HOMA-IR



Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at *p<0.05. The results were [a]compared to control rats and [b]compared to diabetic rats

Figure 13

Effect of metformin-3-hydroxyflavone complex on QUICKI

Table 1

The levels of fasting blood glucose, glycosylated hemoglobin (HbA1c), plasma insulin, C-peptide and urine sugar in control and experimental groups of rats

Groups	Blood glucose (mg/dl)	HbA1c (%)	Insulin (μU/mL)	C-peptide (pmol/mL)	Urine sugar
Control	96.74 ± 5.04	4.52 ± 0.39	15.42 ± 0.27	0.26 ± 0.02	Nil
Diabetic control	267.15 ± 9.87[a*]	9.87 ± 0.53[a*]	7.28 ± 0.43[a*]	0.11 ± 0.07[a*]	>2%
Diabetic + Schiff base complex	116.67 ± 4.67[b*]	6.20 ± 0.72[b*]	12.87 ± 0.56[b*]	0.17 ± 0.01[b*]	Nil
Diabetic + Metformin	108.46 ± 5.31[b*]	5.76 ± 0.87[b*]	13.82 ± 0.45[b*]	0.19 ± 0.01[b*]	Nil

Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at *p<0.05. The results were [a]compared to control rats and [b]compared to diabetic rats.

Table 2

Effect of Schiff base complex on plasma protein and blood urea and serum creatinine levels in experimental group of rats after 30-day treatment

Groups	Plasma protein (g/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)	Serum creatinine (mg/dl)
Control	8.80 ± 0.17	25.12 ± 0.89	2.30±0.09	0.61 ± 0.01
Diabetic control	6.47 ± 0.11[a*]	46.12 ± 1.47[a*]	6.25±0.21 [a]	1.39 ± 0.02[a*]
Diabetic + Schiff base complex	7.43 ± 0.14[b*]	28.30 ± 0.97[b*]	2.72±0.10 [b]	0.76 ± 0.01[b*]
Diabetic + metformin	8.22 ± 0.16[b*]	27.80 ± 0.72[b*]	2.89±0.15 [b]	0.69 ± 0.02[b*]

Values are given as mean ± SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at *p<0.05. The results were [a]compared to control rats and [b]compared to diabetic rats.

Table 3

Effect of Schiff base complex on levels of activities of AST, ALT and ALP in the serum of experimental group of rats

Groups	AST (moles of pyruvate liberated/h/mg of protein)	ALT (moles of pyruvate liberated/h/mg of protein)	ALP (moles of phenol liberated/min/mg of protein)
Control	72.06 ± 2.28	21.37 ± 0.53	72.26 ± 0.86
Diabetic control	130.88 ± 2.27 [a*]	44.25 ± 1.62 [a*]	150.05 ± 1.55[a*]
Diabetic + Schiff base complex	82.84 ± 2.29 [b*]	23.50 ± 1.05 [b*]	78.82 ± 1.07 [b*]
Diabetic + metformin	70.28 ± 1.89 [b*]	22.12 ± 0.84 [b*]	75.75 ± 1.74 [b*]

Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at *p<0.05. The results were [a]compared to control rats and [b]compared to diabetic rats.

Table 4
Levels of lipid peroxides and hydroperoxides in plasma and pancreatic tissues of control and experimental groups of rats

Groups	Lipid peroxides		Hydroperoxides	
	Plasma (nM/ml)	Pancreas (mM/100g wet tissue)	Plasma (10^{-5} mM/dl)	Pancreas (mM/100 g wet tissue)
Control	3.91 ± 0.25	32.49 ± 2.16	11.12 ± 2.04	16.56 ± 1.26
Diabetic	8.75 ± 0.44[a*]	74.75 ± 3.12[a*]	30.39 ± 1.45[a*]	34.36 ± 1.03[a*]
Diabetic + Schiff base complex	4.56 ± 0.26[b*]	42.21 ± 5.53[b*]	15.12 ± 1.32[b*]	22.68 ± 1.35[b*]
Diabetic + Metformin	4.01 ± 0.24[b*]	44.37 ± 4.19[b*]	13.01 ± 1.31[b*]	21.90 ± 1.33[b*]

Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at *p<0.05. The results were [a]compared to control rats and [b]compared to diabetic rats.

Table 5
Effect of Schiff base complex treatment on the plasma levels of non-enzymatic antioxidants in experimental group of rats

Groups	Vitamin C (mg/dl)	Vitamin E(mg/dl)	Ceruloplasmin (mg/dl)
Control	1.51 ± 0.02	0.78 ± 0.03	12.02 ± 0.42
Diabetic	0.35 ± 0.04[a*]	0.38 ± 0.04[a*]	4.68 ± 0.49[a*]
Diabetic + Schiff base complex	0.85 ± 0.02[b*]	0.65 ± 0.03[b*]	9.72 ± 0.81[b*]
Diabetic + Metformin	0.89 ± 0.03[b*]	0.69 ± 0.05[b*]	9.02 ± 0.67[b*]

Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at *p<0.05. The results were [a]compared to control rats and [b]compared to diabetic rats.

Table 6
Effect of Schiff base complex treatment on the activities of pancreatic SOD, CAT, GPx and GST in the experimental group of rats

Groups	SOD	CAT	GPx	GST
Control	5.45 ± 0.19	17.85 ± 0.21	7.25 ± 0.29	6.49 ± 0.35
Diabetic	1.59 ± 0.22[a*]	5.60 ± 0.35[a*]	3.25 ± 0.31[a*]	1.49 ± 0.21[a*]
Diabetic + Schiff base complex	3.02 ± 0.13[b*]	12.12 ± 0.52[b*]	5.75 ± 0.20[b*]	4.58 ± 0.20[b*]
Diabetic + Metformin	3.56 ± 0.12[b*]	13.28 ± 0.49[b*]	6.05 ± 0.50[b*]	4.89 ± 0.19[b*]

SOD: one unit of SOD activity is the amount of enzyme required to inhibit auto-oxidation of epinephrine by 50 %.
CAT: Unit of catalase activity is nmoles of hydrogen peroxide decomposed/min/mg of protein.
GPx: Unit of GPx activity is µmol glutathione oxidized/min/mg of protein.
GST: Unit of GST activity is µmol 1-chloro-2, 4-dinitrobenzene conjugate formed min^{-1} mg^{-1} protein.
Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. Values are statistically significant at *p<0.05. The results were [a]compared to control rats and [b]compared to diabetic rats.

DISCUSSION

The Schiff base ligand (L) was synthesized from the coordination between 3-hydroxyflavone and metformin by a molar ratio method. The Schiff base ligand (L) was formed as a yellow-colored product (Scheme 1). The IR spectral data of metformin hydrochloride, 3-hydroxyflavone and Schiff base L are represented in Figure 1, 2 and 3 respectively. In free metformin, the free amine exhibit $\nu(NH_2)$ a peak around 3162 cm^{-1}. The bands in the region of 3386 cm^{-1} and 3271 cm^{-1} are due to the presence of N–H stretching. The sharp peaks around 2824 cm^{-1} are due to the presence of methyl groups coordinated to nitrogen group and the nitrogen connected with carbon and hydrogen. The peak around 1159 cm^{-1} is due to the presence of carbon–nitrogen stretching as depicted in Figure 1[53.] Likewise, metformin-free 3-hydroxyflavone exhibited various peaks. In the free 3-hydroxyflavone, the peak around 3216 cm^{-1} is due to the presence of the hydroxyl group present in the aromatic ring. The peak around 3089 cm^{-1} is owing to the presence of C–H stretching. The peaks around 1656 and 1582 cm^{-1} is due to the presence of carbonyl and C=C groups. The peaks corresponding to C–O and C–O–C were found in the range of 1359 to 1248 cm^{-1}[154.] The formation of the Schiff base ligand L has been confirmed by infrared spectroscopy. IR bands obtained at 1638 cm^{-1} clearly indicate the presence of an imine functionality which is due to the condensation of 3-hydroxyflavone with metformin. IR band at 3162 cm^{-1} for the NH_2 stretching was not observed which clearly indicate that the primary amine functionality of metformin has successfully condensed with the carbonyl functionality of 3-hydroxyflavone. The other peaks corresponding to various functional groups did not show any alterations which result in the formation of Ligand L.Mass spectrum analysis of Schiff base ligand L is shown in Figure 4. The molecular ion peak [M+] at m/z = 349 confirms the molecular weight of the Schiff base ligand $C_{19}H_{19}N_5O_2$. The peaks at m/z = 273, 245, 195, 167, and 125 correspond to the various fragments of $C_{13}H_{15}N_2O_2$, $C_{11}H_{11}N_5O_2$, $C_7H_9N_5O_2$, $C_7H_9N_3O_2$, and

$C_6H_7NO_2$ respectively. This confirms the molecular structure of the ligand. The ^1H NMR spectra of metformin, 3-hydroxy flavone and Schiff base ligand (L) were shown in Figures 5, 6 and 7, respectively. The ^1H NMR spectrum of synthesized Schiff's base ligand was recorded in DMSO-d_6. In the NMR spectrum of the Ligand L, the signal as singlet in the range δ 1.82 ppm is due to six methyl protons in the metformin. In the aromatic region, a few doublets and in few cases some overlapping doublets/multiplets are observed in the range of δ 7.3–8.5 ppm. The singlet corresponding to phenolic proton was observed at δ 9.35 ppm. The singlets in the region of δ 6.9 and 7.2 ppm were due to the presence of two amide (C=NH) and one C-NH[55, 56.] ^{13}C NMR spectra of metformin, 3-hydroxyflavone and Schiff base ligand (L) are represented in Figures 8, 9 and10**.** The ^{13}C NMR spectrum of the Schiff base ligand exhibited signals in the region of δ 116–155 ppm corresponding to aromatic carbons. The peak around δ172 ppm is due to the presence of C=N carbon[57.] The methylene carbons were found in the region of δ 35 ppm. The amide carbons were observed in the region of δ 156 and 160 ppm[58.] On examining the data obtained through various spectral studies of the Schiff base to that of metformin hydrochloride and 3-hydroxyflavone, it was found that the spectrum of the complex showed data similar to that of individual components with slight modifications in some regions due to the coordination between the metformin hydrochloride and 3-hydroxyflavone. The main shifts account for the complex deformation is in the N=CH stretching region[59.] The spectral data obtained for metformin as well as 3-hydroxyflavone are in accordance with several reports available in the literature[60, 61.] The acute toxicity and the dosage fixation studies were conducted as per OECD guideline (423). Based on the dose-dependent activity of metformin-3-hydroxyflavone complex on fasting blood glucose levels, 20 mg/kg b.w./rat/day for 30 days was fixed as the optimal dosage for evaluating the antidiabetic and antioxidant properties of the complex. High fat diet fed–low dose STZ induced experimental type 2 diabetes in rats is an ideal animal model as it closely resembles the clinical and metabolic characteristics of human type 2 diabetes and is widely used for pharmacological screening[62, 63.] Therefore, it was used in the present study to evaluate the antidiabetic properties of metformin-3-hydroxyflavone complex. Figure 11 shows the effect of oral administration of the metformin-3-hydroxyflavone complex on glucose tolerance in diabetic rats after oral glucose load. The maintenance of normal glucose homeostasis involves the simultaneous and coordinated roles of the pancreatic β-cells, the liver and the peripheral tissues, primarily the skeletal muscle[64.] OGTT is routinely used to evaluate whole body glucose tolerance *in vivo*[65.] Although several attempts have been made to assess insulin sensitivity from the OGTT,[66,67] it has been difficult to derive meaningful information about whole-body, peripheral tissue or hepatic sensitivity to insulin from the results of the OGTT[68, 69.] The product of the glucose area under the plasma glucose curve and insulin under the plasma insulin curve has been used as an index of insulin resistance[70, 71.] Due to peripheral insulin resistance coupled with insufficient levels of insulin secretion, the blood glucose concentration before oral glucose load in the diabetic rats was significantly higher than the glucose levels in control rats. The blood glucose levels reached a maximum peak at 60 min and did not return to the basal level even at the end of 120 min indicating the alterations in glucose homeostasis. Oral administration of the metformin-3-hydroxyflavone complex to diabetic rats showed a significant decrease in blood glucose concentration at fasting stage when compared with diabetic rats. After oral glucose load, the blood glucose levels reached a maximum peak at 60 min but were significantly lower than those in diabetic rats and the peak was reversed to the basal level in the next 60 min. These observations suggest that metformin-3-hydroxyflavone complex possesses the efficacy to maintain glucose homeostasis in hyperglycemic conditions. HOMA-IR and QUICKI values of control and experimental groups of rats are depicted in Figure 12. Due to peripheral insulin resistance, diabetic rats showed a significant elevation of HOMA-IR and the same was decreased significantly upon treatment with oral administration of metformin-3-hydroxyflavone complex as well as metformin. Quantitative insulin check index is depicted in Figure 13. The diabetic rats showed a lesser index as compared to the control rats, indicating that insulin sensitivity was notably reduced in diabetic animals. Oral administration of metformin-3-hydroxyflavone complex restored the insulin sensitivity. The normalization of HOMA-IR and QUICKI indices suggest that metformin-3-hydroxyflavone complex improves insulin sensitivity in diabetic rats. Table 1 depicts the levels of fasting blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin and C-peptide and the inference for the presence of urine sugar in control and experimental groups of rats. Diabetic rats showed significantly elevated levels of fasting glucose and glycosylated hemoglobin and a concomitant decrease in the levels of hemoglobin and the altered levels were significantly reverted back to near normal in metformin-3-hydroxyflavone complex treated diabetic rats. Due to the insulin insufficiency and resistance in diabetic rats, the fasting blood glucose levels were elevated, which ultimately leads to elevated glycosylated hemoglobin levels, because hemoglobin in circulation binds to glucose during persistently elevated fasting blood glucose levels by an irreversible, non-enzymatic manner to form glycosylated hemoglobin[72.] During chronic hyperglycemia, the glycation of hemoglobin at N-terminal valine and numerous lysine residues occurs, which leads to an elevated level of glycosylated hemoglobin with a concomitantly reduced level of hemoglobin. The level of HbA1c reflects the average blood glucose level over the past 3 months and is often considered as a hallmark in the diagnosis and prognosis of diabetes mellitus[73.] The decreased levels of HbA1c evidenced the significant antidiabetic and antioxidant properties of metformin-3-hydroxyflavone complex.Plasma insulin and C-peptide levels in the diabetic rats were markedly reduced when compared with control rats whereas these altered levels were significantly improved in diabetic rats treated with metformin-3-hydroxyflavone complex. Chronic hyperglycemia in diabetic condition induces increased insulin secretion from the functioning pancreatic β cells, ultimately leads to hyperinsulinemia coupled with hyperglycemia. This condition stresses the pancreatic β

cells to produce more insulin, which ultimately resulted in a severe destruction of β cells and responsible for decreased secretion of insulin in diabetic rats[74]. In the insulin synthesis pathway, first preproinsulin was translocated into the endoplasmic reticulum of pancreatic β cells with an A-chain, a C-peptide, a B-chain and a signal sequence. Subsequently, C-peptide was secreted in equimolar amounts to insulin[75]. Urine sugar was present in diabetic rats. However, after 30 days of treatment with metformin-3-hydroxyflavone complex, urine sugar was no longer detected; suggesting metformin-3-hydroxyflavone complex improves the secretion of insulin and C-peptide from the remnant β cells of pancreas to maintain glucose homeostasis in diabetic rats. The antidiabetic efficacy of the complex at a concentration of 20mg/kg.b.w was comparable with metformin which was administered at a relatively higher concentration (50mg/kg.b.w.). The levels of plasma protein, blood urea, serum creatinine and uric acid were depicted in Table 2. Diabetic rats showed decreased plasma protein level with concomitant increased levels of renal tissue markers such as urea, uric acid and creatinine. The levels were significantly reverted back to near normalcy in metformin-3-hydroxyflavone complex treated diabetic rats. Metformin treated diabetic rats also showed similar improvement in the levels of plasma protein, blood urea, serum creatinine and uric acid. Enhanced insulin resistance coupled with increased muscle wasting responsible for increased protein glycation and reduced protein synthesis resulted in decreased plasma protein level in diabetic condition[76]. Decreased protein anabolism with concomitant increased protein catabolism causes remarkable effects in the metabolic functions of renal tissues, which leads to elevated levels of blood urea, serum uric acid and serum creatinine. Protein catabolic end products namely urea, creatine and phosphocreatine breakdown product creatinine, which are considered as energy storage compounds in muscle tissues. The elevated levels of blood urea, serum uric acid and serum creatinine suggest the impairment in the renal function to excrete urea, uric acid and creatinine. The levels of these markers were significantly reduced in metformin -3-hydroxyflavone complex treated diabetic rats, indicating the beneficial effect of the complex in ameliorating diabetes associated renal complications.Table 3 represents the activities of pathophysiological enzymes such as AST, ALT and ALP. Diabetic control rats showed elevated activities of these enzymes when compared with control rats. Measurement of these pathophysiological enzymes is one of the most sensitive and dramatic indicators of hepatocyte injury. The cytosolic enzymes AST and ALT are released into the circulation during cellular damage[77]. During tissue damage, the membrane bound enzyme ALP is released into portal circulation[78]. Significantly elevated activities of these enzymes were indicative of cellular damage and loss of the functional integrity of the cell membranes[79, 80]. The elevated activities of these hepatic marker enzymes AST, ALT and ALP in diabetic rats were significantly reduced upon treatment with the metformin-3-hydroxyflavone complex. This data revealed the non-toxic as well as tissue protective nature of the metformin-3-hydroxyflavone complex. Similar observations were noted in metformin treated diabetic rats also.Table 4 exemplifies the levels of lipid peroxides and hydroperoxides in plasma and pancreatic tissues of control and experimental groups of rats. Increased oxidative stress appears to be a deleterious factor leading to insulin resistance, dyslipidemia, β-cell dysfunction, impaired glucose tolerance and ultimately leading to T2DM and its secondary complications[81]. The increased levels of oxidative stress markers such as lipid peroxides and hydroperoxides accompanied by decreased levels of both enzymatic and non-enzymatic antioxidants[82]. The significant increase noted on the levels of lipid peroxides and hydroperoxides in plasma and pancreatic tissues of the diabetic group of rats were declined to near normalcy by the oral treatment of metformin -3-hydroxyflavone complex as well as metformin to diabetic groups of rats. Table 5 depicts the levels of non-enzymatic antioxidants such as vitamin C, vitamin E and ceruloplasmin in plasma of control and experimental groups of rats. Vitamin C, a hydrophilic antioxidant sequesters the singlet oxygen radical, stabilize the hydroxyl radical and regenerate reduced vitamin E back to the active state[83]. Vitamin E, a lipophilic antioxidant, transfers its phenolic hydrogen to a peroxyl free radical of peroxidized polyunsaturated fatty acids, thereby breaking the radical chain reaction and averting the peroxidation of membrane lipids[84]. Ceruloplasmin is a powerful non-enzymatic antioxidant that inhibits lipid peroxidation by binding with copper[85]. The observed decrease in the levels of oxidative stress markers along with increased levels of both enzymatic and non-enzymatic antioxidants in the complex treated rats clearly evidenced the antioxidant properties of metformin-3-hydroxyflavone complex.Table 6 depicts the activities of enzymatic antioxidants such as SOD, CAT, GPx and GST in pancreatic tissues of control and experimental groups of rats. Enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase form the first line of defense against the generation of ROS in the system; play an important role in scavenging the toxic intermediates of incomplete oxidation[86]. Pancreatic β-cells are vulnerable to oxidative stress because of their diminished activities of free radical quenching enzymatic antioxidants[87]. The activities of enzymatic antioxidants were significantly diminished in the pancreatic tissues of diabetic group of rats. Oral treatment of synthesized complex significantly attenuated the altered activities of these enzymatic antioxidants to near normalcy in pancreatic tissues of the diabetic rats, which in turn reveals the antioxidant potential of metformin-3-hydroxyflavone complex. The antioxidant properties of the complex are mainly attributed to the presence of 3-hydroxyflavone.

CONCLUSION

In conclusion, the results of the present study establishes the pre-clinical efficacy and safety of a new metformin-3-hydroxyflavone complex in ameliorating the primary and secondary complications of type 2 diabetes mellitus. Further studies are in progress to understand the molecular mechanisms involved in the maintenance of euglycemia by metformin-3-hydroxyflavone complex treatment.

ACKNOWLEDGMENTS

The Research Fellowship of the University Grant Commission (UGC), New Delhi, India, to Mr. C. Jaiganesh in the form of UGC-BSR-RF is gratefully acknowledged.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2009; 32(Suppl 1): S62–S67.
2. Kang X, Wang C, Chen D, Lv L, Liu G, Xiao J, Yang Y, He L, Chen L, Li X, Tian H, Jia W, Ran X. Contributions of Basal Glucose and Postprandial Glucose Concentrations to Hemoglobin A1c in the Newly Diagnosed Patients with Type 2 Diabetes—The Preliminary Study. Diabetes Technol Ther. 2015; 17(7): 445–448
3. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001; 414 (6865): 782-787.
4. Thakare V, Shende SS, Shirure PA, Swami OC. Role of conventional oral antidiabetic drugs in management of type 2 diabetes mellitus. Int J Res Med Sci. 2017; 5(3): 749-758.
5. Holman R. Metformin as first choice in oral diabetes treatment: the UKPDS experience. Journ Annu Diabetol Hotel Dieu. 2007:13-20.
6. Bailey CJ, Day C. Metformin: its botanical background. Practical Diabetes International. 2004; 21(3):115–7.
7. Adler AI, Shaw EJ, Stokes T, Ruiz F. Newer agents for blood glucose control in type 2 diabetes: summary of NICE guidance. Guideline Development Group. BMJ. 2009; 22(338): b1668.
8. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B. Medical Management of Hyperglycemia in Type 2 Diabetes: A Consensus Algorithm for the Initiation and Adjustment of Therapy A consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2009; 32(1): 193–203.
9. Senthilkumar KL, Ehizilmuthu RP. Formulation, development and evaluation of metformin hydrochloride sustained release tablets. International Journal of Pharma and Bio Sciences. 2011; 2(2): 77-82.
10. Kumar M, Mandal V, Hemalatha S. Detection of metformin hydrochloride in a traditionally used Indian herbal drug for antidiabetic: a case report. International Journal of Pharma and Bio Sciences. 2011; 2(2): 307-313.
11. Pederson J. The effect of metformin on weight loss in obesity. Acta Endocrinologica. 1965; 49: 479-486.
12. Goodarzi MO and Bryer-Ash M. Metformin revisited: re-evaluation of its properties and role in the pharmacopoeia of modern antidiabetic agents. Diabetes Obes Metab. 2005; 7: 654 –665
13. DeFronzo RA, Barzilai N, Simonson DC. Mechanism of metformin action in obese and lean noninsulin-dependent diabetic mellitus. N Engl J Med. 1995; 333: 550-554
14. Pentikainen P, Neuvonen PJ, Penttila A. Pharmacokinetics of metformin after intravenous and oral administration to man. Eur J C/in Pharmacol. 1979; 16: 195-202
15. Noel M. Kinetic study of normal and sustained release dosage forms of metformin in normal subjects. J mt Biomed Jnf Data. 1980; 1: 9-20
16. Sirtori CR, Franceschini G, Galli-Kienle M, Cighetti G, Galli G, Bondioli A, Conti F, Disposition of metformin (N,N-dimethylbiguanide) in man. Clin Pharmacol Ther. 1978; 24: 683-693
17. Tucker GT, Casey C, Phillips PJ, Connor H, Ward JD, Woods HF. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. Br J C/in Pharmacol. 1981; 2: 235-246.
18. Guntupalli J, Eby B, Lau K. Mechanism for the phosphaturia of NH4Cl: dependence on acidemia but not on diet PO4 or PTH. Am J Physiol. 1982; 242: F552–F560.
19. Nowik M, Picard N, Stange G, Capuano P, Tenenhouse HS, Biber J, Murer H, Wagner CA. Renal phosphaturia during metabolic acidosis revisited: molecular mechanisms for decreased renal phosphate reabsorption. Pflugers Arch. 2008; 457: 539–549
20. Filioussi K, Bonovas S, Katsaros T. Should we screen diabeticpatients using biguanides for megaloblastic anaemia? Aust Fam Physician. 2003; 32: 383–384
21. Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors ofvitamin B(12) deficiency in patients receiving metformin. Arch Intern Med. 2006; 166: 1975–1979.
22. Nye HJ, Herrington WG. Metformin: the safest hypoglycaemic agent in chronic kidney disease? Nephron Clin Pract. 2011;118 (4): 380-383.
23. Goldstein BJ, Feinglos MN, Lunceford JK et al. Effect of initial combination therapy with sitagliptin, a dipeptidyl peptidase-4 inhibitor, and metformin on glycemic control in patients with type 2 diabetes. Diabetes Care. 2007; 30: 1979–1987.
24. Phillips M, Cataneo RN, Cheema T, Greenberg J. Increased breath biomarkers of oxidative stress in diabetes mellitus. Clin Chim Acta. 2004; 344:189-194
25. Niedowicz D, Daleke D. The role of oxidative stress in diabetic complications. Cell Biochem Biophys. 2005; 43: 289-330.
26. Ramakrishna A, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in

plants. Plant Signal Beha. 2011; 6(11): 1720–1731.
27. Pandey A, Tripathi P, Pandey R, Srivatava R, Goswami S. Alternative therapies useful in the management of diabetes: A systematic review. Journal of Pharmacy and Bioallied Science. 2011; 3(4): 504-512.
28. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine.1996; 20: 933–956.
29. Cornard JP and Merlin JC. Comparison of the chelating power of hydroxyflavones. Journal of Molecular Structure. 2003; 651–653: 381–387.
30. Nayak Y, Venkatachalam H, Daroji VK, Mathew G, Jayashree BS, Unnikrishnan MK. Antidiabetic activity of 3-hydroxyflavone analogues in high fructose fed insulin resistant rats. EXCLI J. 2014; 13: 1055-1074.
31. 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. Pharmacological Research. 2005; 52: 313–320.
32. Bartoli E, Fra GP, Schianca C. The oral glucose tolerance test (OGTT) revisited. European Journal of Internal Medicine. 2011; 22: 8–12.
33. Matthews DR. Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28: 412–419.
34. Mather K. Surrogate measures of insulin resistance: of rats, mice, and men. Am J Physiol Endocrinol Metab. 2009; 296(2): 398-399.
35. Trinder P. Determination of blood glucose using an oxidase–peroxidase systemwith a non-carcinogenic chromogen. J Clin Pathol. 1969; 22:158–161.
36. Drabkin DL, Austin JH. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J Biol Chem. 1932; 98: 719–733.
37. Nayak SS, Pattabiraman TN. A new colorimetric method for the estimation of glycosylated hemoglobin. Clin Chim Acta. 1981; 109: 267–274.
38. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193: 265–275.
39. Natelson S, Scott ML, Beffa C. A rapid method for the estimation of urea in biologic fluids. Am J Clin Pathol. 1951; 21: 275–281.
40. Brod J, Sirota JH. The renal clearance of endogenous creatinine in man. J Clin InvesT. 1948; 27:645–654.
41. Caraway WT. Determination of uric acid in serum by a carbonate method. Am J Clin Pathol. 1955; 25: 840–845.
42. King J. Transaminases: alanine and aspartate transaminases. Practical Clinical Enzymology. 1965a; Van D. Nostrand Co., London: 363–395.
43. King J. The hydrolases-acid and alkaline phosphatases. Practical Clinical Enzymology. 1965b; Van D. Nostrand Co., London: 199–208.
44. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351–358.
45. Jiang ZY, Hunt JV, Wolff SP. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem. 1992; 202: 384–389.
46. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol. 1979; 62: 3–11.
47. Desai JD. Methods in enzymology. In: Parker (eds). 1984; Academic Press, New York: 138.
48. Ravin HR. An improved colorimetric enzymatic assay of ceruloplasmin. J Lab Clin Med. 1961; 58: 161–168.
49. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247: 3170–3175.
50. Takahara S, Hamilton HB, Neel JV, Kobara TY, Ogura Y, Nishimura ET. Hypocatalasemia: a new genetic carrier state. J Clin Invest. 1960; 39: 610–619.
51. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973; 179: 588–590.
52. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249: 7130–7139.
53. Sheela NR, Muthu S, Krishnan SS. FTIR, FT Raman and UV-Visible Spectroscopic Analysis on Metformin Hydrochloride. Asian J Chem. 2010; 22: 5049-5056.
54. Uivarosi V, Badea M, Olar R, Draghici C, Barbuceanu SF. Synthesis and Structural Investigation of New Bio-Relevant Complexes of Lanthanides with 5-Hydroxyflavone: DNA Binding and Protein Interaction Studies. Molecules. 2013; 18: 7631-7645.
55. Gadape HH, Parikh KS. Quantitative Determination and Validation of Metformin Hydrochloride in Pharmaceutical Using Quantitative Nuclear Magnetic Resonance Spectroscopy. E-Journal of Chemistry. 2011; 8(2): 767-781.
56. Singh K, Thakur R, Kumar V. Co(II), Ni(II), Cu(II), and Zn(II) complexes derived from 4-[{3-(4-bromophenyl)-1-phenyl-1Hpyrazol-4-ylmethylene}-amino]-3-mercapto-6-methyl-5-oxo-1,2,4-triazine. beni-suef university journal of basic and applied sciences. 2016; 5: 21–30.
57. Neelakantan MA, Esakkiammal M, Mariappan SS, Dharmaraja J, Jeyakumar T. Synthesis, Characterization and Biocidal Activities of Some Schiff Base Metal Complexes. Indian J Pharm Sci. 2010; 72(2): 216–222.
58. Kurzwernhart A, Kandioller W, Bartel C, Bächler S, Trondl R, Mühlgassner G, Jakupec MA, Arion VB, Marko D, Keppler BK, Hartinger CG. Targeting the DNA-topoisomerase complex in a double-strike approach with a topoisomerase

inhibiting moiety and covalent DNA binder, Chem Commun (Camb). 2012; 18;48 (40): 4839-4841
59. Socrates G, Infrared Characteristic Group Frequencies, 1st Ed., John Wiley, New York, 1980.
60. Krishan B, Iqbal SA. Synthesis, Spectral Characterization, Magnetic Moment, Mass, X-Ray Diffraction, and Kinetic (TGA) Studies of Cr(III) Complex with Metformin: An Oral Antidiabetic Drug. Journal of Chemistry Volume. 2014; Article ID 378567: 11.
61. Moissette A, Hureau M, Kokaislova A, Person AL, Cornard JP, Waelea ID, Gener IB, Spectroscopic evidence of 3-hydroxyflavone sorption within MFI type zeolites: ESIPT and metal complexation. Phys Chem Chem Phys. 2015; 17: 26207.
62. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, Reaven GM. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism. 2000; 49: 1390–1394.
63. Pari L, Srinivasan S. Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. Biomedicine & Pharmacotherapy. 2010; 64(7): 477–481.
64. Wilcox G. Insulin and Insulin Resistance. Clin Biochem Rev. 2005; 19(2): 26-39.
65. Preethi BL, Jaisri G, Kumar KM, Sharma R. Assessment of insulin resistance in normoglycemic young adults. Fiziol Cheloveka. 2011; 37(1): 25-118.
66. Bergman R, Finegood D, Ader M. Assessment of insulin sensitivity in vivo. Endocrino Rev. 1985; 6: 45-86.
67. Matsuda M, DeFronzo R. In vivo measurement of insulin sensitivity in humans. In Clinical Research in Diabetes and Obesity: Part I: Methods, Assessment, and Metabolic egulation. Draznin B, Rizza R, Eds. Totowa NJ, 1997; Humana Press: 23–65.
68. Matsuda M, DeFronzo RA. Insulin Sensitivity Indices Obtained From Oral Glucose Tolerance Testing. Diabetes Care. 1999; 22: 1462–1470.
69. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, YkiJarvinen H, Haeften TV, Renn W, Gerich J. Use of the oral glucose tolerance test to assess insulin releaseand insulin sensitivity. Diabetes Care 2000; 23: 295-301.
70. Levine R, Haft D. Carbohydrate homeostasis. N Engl J Med. 1970; 283: 237-246.
71. Myllynen P, Koivisto V, Nikkila E. Glucoseintolerance and insulin resistance accompany immobilization. Acta Med Scand. 1987; 75:81-222.
72. Clark SL, Santin AE, Bryant PA, Holman R, Rodnick KJ. The initial noncovalent binding of glucose to human hemoglobin in nonenzymaticglycation. Glycobiology. 2013; 23(11): 9-1250.
73. Florkowski C. HbA1c as a Diagnostic Test for Diabetes Mellitus. ClinBiochem Rev.2013; 34(2): 75–83.
74. Wajchenberg B. Beta-cell failure in diabetes and preservation by clinical treatment. Endocrine Rev. 2007; 28: 187-218.
75. Prentki M, Nolan JC. Islet β cell failure in type 2 diabetes. J Clin Invest. 2006; 116(7): 1802-1812.
76. Cerf ME. Beta Cell Dysfunction and Insulin Resistance. Front Endocrinol (Lausanne). 2013; 4: 37.
77. Sallie R, Tredger JM, Williams R. Drugs and the liver. Part 1: testing liver function. Biopharm Drug Dispos. 1991; 12: 251-259.
78. Rajagopal SK, Manickam P, Periyasamy V, Namasivayam N. Activity of Cassia auriculata leaf extract in rats with alcoholic liver injury. J Nutr Biochem. 2003; 14: 452-458.
79. Saraswat B, Visen PK, Patnaik GK, Dhawan BN. Anticholestatic effect of picroliv, active hepatoprotective principle of Picrorhizakurrooa, against carbon tetrachloride induced cholestasis. Indian J Exp Biol. 1993; 31: 316-318.
80. Rajesh G, Latha MS. Preliminary evaluation of the antihepatotoxicactivity of Kamilari, a polyherbal formulation. J Ethnopharmacol. 2001; 91; 99-104.
81. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes. 2015; 6(3): 456–480.
82. Hassan HA, Agmy SME, Gaur RL, Fernando A, Raj MHG, Ouhtit A. In vivo evidence of hepato-and-reno-protective effect of garlic oil against sodium nitrite induced oxidative stress. Int J Biol Sci. 2009; 5(3): 249-255.
83. Azqueta A, Costa S, Lorenzo Y, Bastani NE, Collins AR. Vitamin C in cultured human (HeLa) cells: lack of effect on DNA protection and repair. Nutrients. 2013; 5(4): 1200-17.
84. Marcadenti A, Coelho RCLA. Dietary Antioxidant and Oxidative Stress: Interaction between Vitamins and Genetics. J Nutrition Health Food Sci. 2015; 3(1): 1-7.
85. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behaviour of flavonoides: structure-activity relationships. Free Radic Bio Med. 1997; 22: 749-760.
86. Rodrigo R. Prevention of postoperative atrial fibrillation: novel and safe strategy based on the modulation of the antioxidant system. Frontiers in Physiology. 2012; 3: 93.
87. Robertson RP, Harmon J, Tran POT, Poitout V. β-Cell Glucose Toxicity, Lipotoxicity, and Chronic Oxidative Stress in Type 2 Diabetes. Diabetes. 2004; 53(1): S119- S124.

Reviewers of this article

Asst.Prof.Dr. Sujata Bhattachary

Assistant Professor, School of Biological and Environmental Sciences, Shoolini University, Solan (HP)-173212, India

Dr K Rajagopal,M.Sc.,M.Phil.,Ph.D

Head, Dept of Biotechnology,Vels University,Pallavaram,Chennai,India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript