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RESEARCH ARTICLE

Evaluation of antidiabetic and antioxidant potential of some novel ethoxylated head of α, β-Unsaturated ketones

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ABSTRACT:
The aim of this study is to evaluate the *in vivo* antidiabetic and *in vitro* antioxidant activity of newly synthesized α, β-unsaturated ketones with ethoxy head. Eleven newly synthesized compounds (E1 - E11) were taken to evaluate for their *in vitro* antioxidant activity by DPPH and hydrogen peroxide assay method and six compounds which contain electron donating groups were selected for screening of *in vivo* antidiabetic potential by high fat diet, streptozotocin induced diabetes in Albino Wistar rats. The compounds containing electron donating groups were found to be good antioxidants when compared to standard ascorbic acid. All the selected compounds were found to be effective for antidiabetic activity. Amongst them compound **E4** shows more prominent antidiabetic activity when compared to standard metformin.

KEYWORDS: α, β-unsaturated ketones, Electron donating groups, Electron withdrawing groups, Antidiabetic activity, Antioxidant activity.

INTRODUCTION:
Chemically chalcones are α, β-unsaturated ketones which play a major role in designing and synthesis of new chemical entity (NCE). Chalcones are synthesized by Claisen–Schmidt condensation which involves a reaction between aromatic aldehydes and ketones in presence of base followed by dehydration. These chalcones nucleus are abundant in edible plants and are considered to be the precursors for biosynthesis of flavonoids and isoflavonoids. This nucleus is found in many natural products mainly in flavonoid family. It is also an important intermediate for many heterocyclic compounds such as pyrazolines, thiazines, oxazines and isoxazolines etc.[1-6] Many flavonoids and other compounds which contain this moiety have been reported with a wide spectrum of biological activities.[7-20]

Number of diseases are reported to be due to the chemicals which induce oxidative stress were not balanced with antioxidant homeostatic phenomenon in our body. Due to the depletion of dietary antioxidants and elevated level of oxidative stress pronounced the generation of free radicals which can leads to many pathological conditions in the body.[21] Free radicals play a major role in many diseases like heart disease, cancer, diabetes, strokes, Parkinson's diseases and allergies.[22]

Oxidative stress contributes in the development of diabetes mellitus (DM) and also shows important role during DM, including diminishing of insulin action and raise of the complication rate. Antioxidants have already shown to be potential in the treatment of DM both type 1 and type 2. Most of the studies have shown relationship between oxidative strain and DM along with their impediments related to heart, liver, kidney and eye. Thus, oxidative stress seems to be more worrying in metabolic disorders of type 2 diabetes. Antioxidants are preventing the development of chronic diseases such as cancer, diabetes and cardiovascular diseases.[23]

DM is a progressive disorder characterized by hyperglycaemia associated with malfunctioning of the

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vital organs of the body due to biochemical alterations of glucose, lipid and protein metabolism.[24] Hyperglycemia generates reactive oxygen species (ROS), which can leads to the destruction of normal function of cells. Earlier literature states that diabetes mellitus induced by streptozotocin (STZ) in experimental animals, resembles type 2 diabetes in humans.[25] Many molecules which contain chalcones as a basic structure have been reported to be effective antioxidants[26,27] and antidiabetic agents.[28-30]

Therefore in the present study, all the newly synthesized compounds are evaluated for antioxidant potential by DPPH (2,2'-diphenyl-1-picrylhydrazyl) and hydrogen peroxide (H_2O_2) assay methods. The chalcones with electron donating groups (EDG) possess enhanced antidiabetic activity when compared to other substituents.[31] Therefore among all the synthesized compounds, only the compounds with EDG (E1, E2, E3, E4, E9 and E10) were selected for their investigation of antidiabetic potentials by HFD-STZ induced diabetics in Albino Wistar rats. Graphical abstract of the present study is given in Figure. 1.

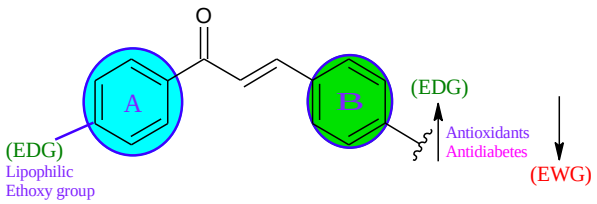

Figure 1: Graphical abstract of the study

MATERIALS AND METHODS:

Chemicals and reagents:

All the chemicals and reagents used in this study are of analytical grade. Streptozotocin and other chemicals used for this study were purchased from Sigma-Aldrich. Disodium hydrogen phosphate and potassium dihydrogen phosphate were obtained from Loba Chemie Pvt. Ltd, Mumbai, India. Metformin was procured as marketed formulation from Sun Pharmaceuticals, Mumbai, India.

Acute toxicity evaluation:

The acute toxicity evaluation was performed according to OECD-423 guidelines and used for the estimation of LD_{50} of synthesized compounds for biological evaluation. [32-34] The Swiss albino mice (n = 6) of either sex were selected randomly and fasted for 24 h with free access to water only. The different synthetic compounds were suspended in normal saline: Tween 80 (95:5) which was administered orally at the dose of 5 mg/kg b.w initially and the incidence of mortality were observed for three days. Further the study was repeated with higher doses such as 50, 300 and 2000 mg/kg. b.w. From acute toxicity study, it was observed that there were no signs and symptoms of acute toxicity at the doses used. Therefore the antidiabetic activity of synthesized compounds was carried out at the dose of 200 mg/kg b.w. (i.e 1/10 of the 2000 mg/kg b.w.) as per OECD guidelines.

Animals:

Healthy adult male Wistar rats weighing between 160 – 190 gm were used for the antidiabetic studies. The experiments were carried out after the due approval from Institutional Animal Ethics Committee (IAEC), Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore. IAEC guidelines were followed for the maintenance of animals and the research work was approved by IAEC No. 115/1611/CPCSEA.

Synthesis and characterization of ethoxylated head of α, β-unsaturated ketones:

Eleven different compounds of α, β-unsaturated ketones with ethoxy head were obtained from the reactions of 4-ethoxyacetophenone and different *para* substituted benzaldehydes in presence of base and ethanol by Claisen-Schmidt method. Synthesis and characterization of these compounds were published by B Lakshminarayanan et al in 2019[35] and depicted in Figure. 2. All the 11 compounds (E1 to E11) were selected for *in vitro* antioxidant activity and the compounds of α, β-unsaturated ketones with ethoxy group at one end and electron donating group on the other end (E1, E2, E3, E4, E9 and E10) were selected for their *in vivo* antidiabetic potential.

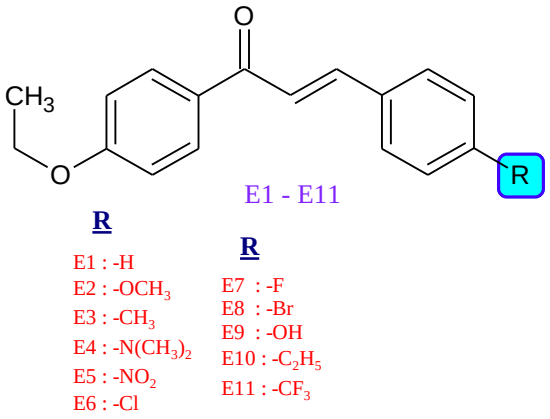

Figure 2: Structures of ethoxy substituted chalcones

In vitro antioxidant activity

DPPH free radical scavenging activity:

All the 11 compounds were selected for *in vitro* antioxidant activity by scavenging of DPPH free radical.[36] Different concentrations of standard ascorbic acid and synthesized compounds (10, 20, 30, 40 and 50 µg/ml) were prepared from stock solution (100µg/ml)

using distilled methanol. One ml of each compound solution was taken in individual test tubes; 4 ml of 0.004% methanol solution of DPPH was added and shaken vigorously. After 30 min of incubation in dark at room temperature, the absorbance was measured against a blank at 517 nm using UV – VIS spectrophotometer (Shimadzu UV-1800) and the percentage radical scavenging activity was calculated by using the formula $[(A_0 – A_1)/A_0] \times 100$, where A_0 is the absorbance of blank, A_1 is the absorbance of the synthesized compounds/standard. The graph was plotted between percent inhibition and concentrations of the sample/standard to obtain the amount of antioxidant necessary to decrease 50% from the initial concentration of DPPH free radicals. IC_{50} values were calculated from calibration curve and defined as the concentration of test/standard compound required to achieve half maximal inhibition.[37-39]

Hydrogen peroxide (H_2O_2) radical scavenging activity:

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS) (pH 7.4). Different concentrations of standard ascorbic acid and synthesized compounds (10, 20, 30, 40 and 50 µg/ml) were prepared from stock solution (100µg/ml) using distilled methanol. One ml of each compound solution was taken in individual test tubes; 2 ml of hydrogen peroxide solution in PBS added to all the test tubes. Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide.[40,41] The percentage scavenging activity and IC_{50} values were measured and calculated by using the formula mentioned in DPPH assay method.

***In vivo* antidiabetic activity of various compounds with EDG using HFD-STZ induced diabetics in rats**

Anti-diabetic activity of synthesized compounds with EDG (E1, E2, E3, E4, E9 and E10) is tested as per the standard method described[42] by using male Wistar rats (160 – 190 g).

Induction of diabetes

High fat diet (HFD) was freshly prepared every day as per the method of Devi et al. [43] Control animals were provided with normal pellet chow (Lipton, India). After 3 days on high fat diet, animals were fasted overnight and diabetes is induced by STZ (50 mg/kg b.w.) intravenously. The STZ was freshly dissolved in citrate buffer (0.01 M/L, pH 4.5) and kept on ice prior to use. One week after STZ administration, the rats with fasting blood glucose concentrations of over 200 mg/dl were considered to be diabetic and were used in the experiments.

Experimental design:

Animals were divided into nine groups of six (n=6) in each. Group 1 treated as normal control (vehicle) received 1.0 ml of 0.5% CMC. Group 2 treated as diabetic control. Group 3 treated as diabetic animals received metformin (50 mg/kg b.w.). Groups 4 to 9 diabetic animals received various compounds of E1, E2, E3, E4, E9 and E10 in a single dose of 200 mg/kg b.w. per oral respectively for 7 days continuously. Body weight, food and water intake were monitored daily for 21 days. Blood was withdrawn from the retro orbital vein each time and blood glucose was measured at 0, 7th, 14th and 21st day by blood glucose monitoring system.

Statistical Analysis:

Values are expressed as mean±SEM. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests using Instat-3 software package (Graph pad), Prism Ltd, USA and values at $p<0.05$ were considered as significant.

RESULTS:

***In vitro* antioxidant activity:**

The scavenging of DPPH radical by synthesized compounds and standard ascorbic acid was determined by decrease in absorbance at 517 nm. The DPPH radical scavenging activity and IC_{50} values were recorded for each compound and shown in the Table 1.

Hydrogen peroxide free radicals are not very reactive, but sometimes it is toxic to cell because it may give rise to hydroxyl radical in the cells. Therefore removing of hydrogen peroxide free radicals is very important for antioxidant defence in cell. The H_2O_2 free radicals scavenging ability and IC_{50} values of synthesised compounds and standard were given in Table 2 and compared with standard ascorbic acid.

Table 1: Antioxidant activities of synthesized compounds (E1 – E11) using DPPH radical scavenging method.

Compo-unds	DPPH radical scavenging activity %					IC_{50} (µg/ml)
	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	
E1	35.24	42.13	49.51	50.29	50.19	41.97
E2	48.93	54.27	56.01	60.09	62.81	10.45
E3	43.49	49.51	52.13	55.04	57.37	25.69
E4	50.29	55.43	59.32	63.10	67.28	8.34
E5	34.27	35.53	36.31	35.63	42.23	112.62
E6	32.23	33.49	34.56	36.01	36.89	172.00
E7	18.54	20.19	21.84	22.13	22.91	320.90
E8	20.58	22.52	23.00	23.98	24.17	371.87
E9	45.92	54.85	57.47	60.87	61.06	13.50
E10	45.63	49.12	52.42	55.92	58.54	23.31
E11	22.42	21.65	24.85	25.53	26.89	246.50
Ascorbic acid	55.53	61.94	65.14	69.80	71.06	7.94

Table 2: Antioxidant activities of synthesized compounds (E1 – E11) using H_2O_2 radical scavenging method.

Comp-ounds	H_2O_2 radical scavenging activity %					IC_{50} (µg/ml)
	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	
E1	31.92	36.87	37.32	39.47	41.00	91.40
E2	49.91	50.98	52.42	54.67	56.74	13.35
E3	39.02	42.62	44.60	46.13	48.02	57.58
E4	52.24	53.05	54.04	55.93	56.20	10.05
E5	17.17	19.33	21.85	21.94	21.85	301.54
E6	12.05	13.30	14.92	14.83	14.83	545.00
E7	10.70	10.88	11.33	12.41	14.65	453.77
E8	10.52	11.87	12.32	12.85	12.94	793.00
E9	45.77	48.38	52.24	53.68	54.31	26.54
E10	43.61	45.05	49.55	52.15	54.40	34.50
E11	8.99	7.19	10.52	12.32	14.02	292.99
Ascorbic acid	52.78	55.48	56.11	57.19	60.16	8.75

Antioxidant results revealed that among all the tested compounds, E4, E2 and E9 with an IC_{50} value of 8.34, 10.45 and 13.50 respectively showed good antioxidant activity which is compared to standard ascorbic acid (IC_{50} value of 7.94) in DPPH assay. Also with H_2O_2 free radical scavenging method it was found that E4 and E2 has good antioxidant activity with IC_{50} values of 10.05 and 13.35 respectively when compared to standard ascorbic acid (IC_{50} value of 8.75).

***In vivo* antidiabetic activity:**

The selected synthesized compounds were screened for *in vivo* antidiabetic activity by streptozotocin induced diabetic model in Albino rat. The changes in body weight, water and food intake in STZ induced diabetic rats before and after oral treatment with vehicle, various compounds and metformin for 3 weeks were given in Table 3. Effect of various compounds with EDG and metformin on the blood glucose level in STZ induced diabetic rats were presented in Table 4.

The body weight of all the tested compounds and metformin were increased when compared to diabetic control. Body weight of E4 and metformin treated group was much higher when compared to that of the same before the treatment. Food and water intake of all the tested groups and metformin treated group were decreased when compared to that of the same before the treatment. Metformin and E10 treated groups showed much difference of water intake when compared to before the treatment. Water intake of all the compounds and metformin treated groups showed significant except E1 treated group when compared to diabetic control. On the other hand food intake of metformin and E4 treated groups showed significant when compared to diabetic control.

From the results, compound E4 showed high, E10 and E3 showed moderate significant activity when compared with standard. The percentage antidiabetic activity of the compounds E4, E10 and E3 have 51.34%, 45.31% and 41.26 % respectively when compared to standard metformin which has 58.35 % activity. Other compounds E1, E2 and E9 have less significant activity when compared to standard metformin.

Table 3: Body weight, water and food intake in STZ induced diabetic rats before and after oral treatment with vehicle, various compounds and metformin for 3 weeks.

Treatment / Groups	Body weight (gm)		Water intake(ml/100 g b.w. of rat/day)		Food intake (g/100 g b.w. of rat/day)	
	Before	After	Before	After	Before	After
Normal	179.05 ± 2.18	183.06 ± 3.18	166.08 ± 4.51	170.02 ± 1.43	32.33 ± 1.08	34.46 ± 0.19
Diabetic	179.18 ± 4.02	158.19 ± 1.09	158.19 ± 1.96	179.12 ± 2.35	33.46 ± 3.19	38.48 ± 2.18
E1	161.04 ± 2.43	166.23 ± 1.95*	172.97 ± 3.52	163.35 ± 5.64	41.14 ± 3.46	38.13 ± 4.15
E2	174.48 ± 2.65	176.08 ± 3.46*	167.36 ± 2.46	156.46 ± 3.69*	41.42 ± 4.48	36.14 ± 1.87
E3	182.63 ± 1.35	188.02 ± 4.38*	162.35 ± 2.87	160.67 ± 2.86*	34.46 ± 1.95	32.31 ± 4.06
E4	160.18 ± 3.02	168.18 ± 1.07*	163.12 ± 2.21	152.19 ± 1.86*	42.28 ± 2.31	31.46 ± 0.17*
E9	169.15 ± 3.03	171.21 ± 1.08*	156.29 ± 1.83	149.12 ± 2.45*	36.38 ± 2.38	32.36 ± 3.21
E10	176.13 ± 1.85	181.04 ± 2.31*	162.15 ± 5.64	122.97 ± 3.52*	40.14 ± 3.25	39.23 ± 2.5
Metformin	186.35 ± 2.46	198.43 ±3.86*	159.26 ± 3.76	121.02 ± 3.57*	46.14 ± 3.19	30.08 ± 0.98*

Values are expressed as mean±SEM (n=6). *P < 0.05, compared to diabetic control group

Table 4: Effect of various compounds with EDG on blood glucose level in STZ induced diabetic rats.

Treatment / Group	Fasting blood glucose (mg/dl)				% Activity
	0 day	7 day	14 day	21 day	
Normal control	75.6 ± 8.6	77.8 ± 1.6	76.4 ± 2.3	78.6 ± 1.4	---
Diabetic control	257.4 ± 4.1	259.7 ± 3.6	256.4 ± 4.6	259.7 ± 3.8	---
E1 (200 mg/kg b.w.)	262.3 ± 6.4	246.4 ± 6.2	226.5 ± 2.3	216.5 ± 4.4	15.52
E2 (200 mg/kg b.w.)	276.7 ± 3.5	258.5 ± 4.8	229.6 ± 4.7	204.6 ± 5.2	20.54
E3 (200 mg/kg b.w.)	271.8 ± 5.6	218.4 ± 6.7*	191.3 ± 4.1*	151.1 ± 2.8*	41.26
E4 (200 mg/kg b.w.)	274.6 ± 6.3	233.5 ± 4.8*	198.8 ± 5.4*	128.6 ± 3.9*	51.34
E9 (200 mg/kg b.w.)	268.8 ± 4.6	239.4 ± 5.7	223.3 ± 7.2	201.1 ± 3.8	17.06
E10 (200 mg/kg b.w.)	269.9 ± 6.4	201.7 ± 5.2*	186.2 ± 3.8*	139.6 ± 5.7*	45.31
Metformin (50 mg/kg b.w.)	265.4 ± 2.3	196.7 ± 4.7*	154.5 ± 3.9*	106.4 ± 2.7*	58.35

Values are expressed as mean±SEM (n=6). *P < 0.05, compared to diabetic control group.

DISCUSSION:

Tested compounds, E4, E2 and E9 showed good antioxidant activity when compared to standard ascorbic acid in DPPH assay and H_2O_2 free radical scavenging method. The better antioxidant activities of E4, E2 and E9 may be due to the presence of electron donating – $N(CH_3)_2$, OCH_3 and OH group on B ring. On the other hand, compounds E1, E3, E10 in the order of E10>E3>E1 showed moderate antioxidant activity in both DPPH and hydrogen peroxide assay methods. Other compounds like E5, E6, E7, E8 and E11 showed low antioxidant activities. The low activities of these compounds may be due to the presence of electron withdrawing NO_2, Cl, F, Br and CF_3 groups on B ring. The parent compound E1 which has no substitution on B ring showed lower antioxidant activity when compared to substitution with EDG on B ring. On the other hand E1 showed better activity then the compound with EWG on B ring.

Nowadays management of diabetes is a need for everyone and it is the challenge in the medical community. STZ is widely used as an agent for the development of experimental diabetes to induce selective dysfunctioning of pancreatic β-cells and the destruction is almost complete.[44,45] The cytotoxic action of STZ is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of β-cells.[46]

The present study revealed that the synthesized compound E4 have highly, E10 and E3 have moderate and E1, E2 and E9 have less significant antidiabetic activity when compared to standard. The percentage increase of blood glucose level was found to be in the order of E1 > E9 > E2 > E3 > E10 > E4 > Metformin. Synthesized compound of α, β-unsaturated ketone with ethoxy group at A ring and dimethyl amino group on B ring (E4) showed high activity than other group attached in ring B. Compounds end with ethyl (E10) and methyl group (E3) also possess a significant antidiabetic activity when compared to standard metformin. Ethoxy head of α, β-unsaturated ketone with substitution at para position in ring B showed increased activity when compared to unsubstituted parent compound (E1). Compounds which contains electron donating group increased the electron density of the molecule and it may help to transport the glucose to muscle and adipose tissue from blood by enhancing the synthesis of glucose transporter or by stimulating the release of insulin into the blood stream and increases glucose utilisation in muscles.[31] It is interesting to note that the antidiabetic activity increases with increasing electron donating capacity of the substituting group in ring B.

CONCLUSION:

In conclusion, results of antioxidant activities of synthesized compounds by DPPH and hydrogen peroxide assay methods confirms that the compounds with electron donating groups possess good antioxidant activity than the compounds with electron withdrawing groups when compared to standard. The results of the antidiabetic study revealed that the compounds tested showed moderate to good antidiabetic activity depends on the electron donating group attached to it.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

ABBREVIATIONS:

NCE: New chemical entity; **DM:** Diabetes mellitus; **HFD:** High fat diet; **ROS:** Reactive oxygen species; **STZ:** Streptozotocin; **DPPH:** 2,2'-diphenyl-1-picrylhydrazyl; **H_2O_2:** Hydrogen peroxide; ***EDG:*** *E*lectron donating group; ***EWG:*** *E*lectron withdrawing group; **OECD:** Organization of economic co-operation and development; **LD_{50}:** Lethal dose 50; **IAEC**: Institutional animal ethics committee; **CPCSEA:** *Committee for the purpose of control and supervision of experiments on animals;* **IC_{50}:** Inhibitory concentration 50; **PBS:** Phosphate buffer Saline; **CMC:** Carboxy methyl cellulose; **SEM:** Standard error of the mean; **ANOVA:** Analysis of variance; **FBG:** Fasting blood glucose.

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