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Research Article

# ANTIDIABETIC EFFECTS OF A SERIES OF CURCUMIN PYRAZOLES *IN-VITRO*

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**ABSTRACT**

**Objective:** To investigate the antidiabetic potential of a series of curcumin pyrazole derivatives *in-vitro*.

**Methods:** The *in-vitro* antidiabetic ability of curcumin pyrazole derivatives (3a-3e) was evaluated by the inhibition studies of the digestive enzymes; including alpha-amylase, rat intestinal alpha-glucosidase, and sucrase. The glucose lowering effect of the compounds was further assessed by the glucose uptake assay using a porcine diaphragm.

**Results:** The curcumin pyrazole derivatives (3a-3e) showed a prominent inhibition of the enzymes studied. Compounds 3a and 3b were found to be more pronounced in their hypoglycemic effects and 3b exhibited the maximum enzyme inhibitory activity. The glucose uptake through porcine diaphragm was extensively promoted by the compounds 3a and 3b than the other compounds in the series.

**Conclusion:** The results suggest that the pyrazole derivatives of curcumin 3a and 3b have potent hypoglycemic properties. These compounds in the series of curcumin pyrazole derivatives (3a-3e) can be selected for further *in-vitro* and *in vivo* studies.

**Keywords:** Diaphragm, Insulin, Hypoglycemic, Enzyme inhibition.

## INTRODUCTION

Diabetes mellitus (DM) is chronic disorder that predominantly alters the metabolism of carbohydrates and that of lipids and proteins too [1]. DM is characterized by an increased blood glucose level. Type I DM is due to an absolute deficiency in the secretion of insulin, whereas Type II DM is more common and is attributable to both resistance to insulin action and impaired insulin secretion [2]. The increasing prevalence of diabetes made an impact in discovering various drugs for the treatment. The majority of the antidiabetic drugs available are either directly or indirectly obtained from the plants. Around 800 plants are reported to possess antidiabetic activity [3-5]. Curcumin is a polyphenol and is the major active component of the rhizome of the plant *Curcuma longa*, commonly called as turmeric. Curcumin had been shown to possess a wide range of biological properties [6]. It has exhibited antidiabetic properties in various models [7]. Though it has a predominant role in the field of medicine, the bioavailability is the major setback for the curcumin [8]. To overcome this limitation, various curcumin derivatives were synthesized and screened for their biological potency [9-11].

In our earlier studies, ethanone pyridine curcumin analogs and cyclopropoxy curcumin analogs were synthesized, and they demonstrated anti-angiogenic and growth suppressing effects on the mouse tumor model [12,13]. A series of curcumin pyrazole derivatives (3a-3e) were synthesized and were shown to possess free radical scavenging activities *in-vitro* [14]. This was the rationale for the continuation of our work on pyrazole derivatives of curcumin since oxidative damage is a root cause for various degenerative diseases in the human system, including Alzheimer's disease, ageing, cancer, cardiovascular diseases, inflammatory disorders, and diabetic complications [15-19].

In the present work, the compounds (3a-3e) were investigated for the *in-vitro* antidiabetic properties by the enzyme inhibition studies and by the glucose uptake assay using the porcine diaphragm.

## METHODS

### Materials

The enzyme alpha-amylase (EC 3.2.1.1) (Type IIA from *Bacillus* species)media were obtained from Sigma-Aldrich Co, Bengaluru, India. p-nitrophenyl-α-D glucopyranoside (PNDG) was procured from SRL, India. Insulin was purchased from Torrent Pharmaceuticals Ltd., under the license from Novo Nordisk, India. The glucose oxidase-peroxidase (GOD-POD) reagent was obtained from Aspen Laboratories, India. All chemicals and solvents are of analytical grade and were purchased from Himedia chemicals, Mumbai, India.

## METHODS

### Glucose uptake assay by porcine diaphragm

Glucose uptake studies were done using the porcine diaphragm with minor modifications [20]. The porcine diaphragm was obtained from a slaughterhouse and washed thoroughly using ice cold saline to get rid of the blood spots. The diaphragm was used to explore the inhibitory effects on the glucose uptake process by curcumin pyrazole derivatives. Diaphragm weighing around 100-150 mg was suspended in a 24 well culture plate containing 0.5 ml saline. 0.2% glucose was added to each well to initiate the response. Dimethyl sulfoxide treated plates served as control. 0.4 units of insulin were used. The volume was made up to 2 ml with saline. Curcumin and curcumin pyrazole derivatives (3a-3e) at 400 and 800 µmol/l concentrations, respectively, were used for the inhibition studies. Plates were incubated for 30 minutes at 37°C in an atmosphere of 100% $O_2$ with shaking. The amount of glucose in the culture plate was determined using the GOD-POD method.

### Alpha-amylase inhibition assay

The alpha-amylase inhibition studies were conducted according to the method described [21]. The enzyme alpha-amylase (1 unit/ml) from *Bacillus* species was dissolved in 0.1 M phosphate buffered saline (pH 6.9). The different concentrations of the curcumin pyrazole derivatives (3a-3e) were pre-incubated with the Curcumin and, enzyme solution for 10 minutes at 37°C. The reaction was initiated by the addition of starch solution (0.1%) to the incubation medium and allowed for the enzymatic reaction for 30 minutes at 37°C. The reaction was terminated by the addition of DNS reagent to the reaction mixture, and the tubes are kept in boiling water bath for 10 minutes. The color obtained was stabilized by the addition of 40% sodium potassium tartarate solution and cooled to room temperature. The optical density was measured at 540 nm. Acarbose was used as positive control. The percentage inhibitory effect of the compounds was calculated by the formula:

$$\%\,\text{Inhibition} = \frac{(\text{control absorption} - \text{sample absorption})}{(\text{control absorption})} \times 100$$

**Preparation of crude enzymes**

The crude enzymes were prepared according to the method described by slight modifications [22]. Briefly, the male rats were sacrificed by cervical dislocation. The intestine was excised and washed with maleate buffer of pH 6 (0.1 M). The inner surface of the intestine containing brush border was carefully scraped and homogenized in maleate buffer in cold. The homogenate obtained was centrifuged for 15 minutes at 1000 g at 4°C. The supernatant was collected and stored at −20°C. This was used as an enzyme source of sucrase and alpha-glucosidase.

**Intestinal alpha-glucosidase and sucrase inhibition assay**

The inhibitory studies of the curcumin pyrazole derivatives against rat intestinal alpha-glucosidase and sucrase were performed according to the method described with few modifications [23]. Briefly, for glucosidase inhibition assay, the crude enzyme solution was pre-incubated with Curcumin and, curcumin pyrazole derivatives (3a-3e) at different concentrations (50-200 µmol/l) for 10 minutes at 37°C. The reaction was initiated by the addition of PNDG solution in maleate buffer (0.1 M, pH 6). The reaction mixture was incubated for 30 minutes at 37°C. The reaction was terminated by the addition of 2 M NaOH solution. The activity of the enzyme was determined at 400 nm using Shimadzu UV-1800 spectrophotometer. Acarbose was used as positive control. The percentage inhibitory effect of compounds was calculated by the formula:

$$\%\,\text{Inhibition} = \frac{(\text{control absorption} - \text{sample absorption})}{(\text{control absorption})} \times 100$$

The inhibitory activity of curcumin pyrazole derivatives against sucrase was determined by assessing the quantity of glucose hydrolyzed from sucrose. Briefly, the crude enzyme solution was pre-incubated with curcumin pyrazole derivatives (3a-3e) at different concentrations (50-200 µmol/l) in maleate buffer (0.1 M, pH 6) for 10 minutes at 37°C. The reaction was initiated by the addition of sucrose solution (60 mmol/l). The reaction mixture was incubated for 30 minutes at 37°C. After incubation, the reaction was terminated by keeping the mixture in a water bath for 10 minutes. The quantity of glucose released in the reaction mixture was set using the GOD-POD method as identified under.

**Estimation of glucose by GOD-POD method**

The glucose produced in the reaction mixture was estimated by the GOD-POD assay kit protocol. In brief, 50 µl of the incubated medium was transferred in a 96 well ELISA plate. The GOD-POD color reagent (200 µl) was added to each well and incubated for the color formation in the dark at 37°C for 30 minutes. The optical density was measured at 505 nm. The percentage yield of glucose was estimated by applying the convention:

$$\%\,\text{glucose production} = \frac{(\text{glucose in control} - \text{glucose in sample})}{(\text{glucose in control})} \times 100$$

**Statistical analysis**

The data obtained were analyzed using MS-Excel software. The data were expressed as mean±standard error of mean, and all the experiments were compared with control and performed in triplicates.

**RESULTS**

The antidiabetic potential of curcumin pyrazole derivatives (3a-3e) was determined by glucose uptake studies using the porcine diaphragm, and the results are depicted in Table 1. The uptake of glucose was found to be increased in the compound treated plates at both 400 µmol/l and 800 µmol/l concentrations, respectively, in comparison with the control. Compound 3a, 3b, and 3e exhibited prominent activity. The increase in the uptake of glucose was also observed in the compound 3c and 3d incubated plates. The absorption of glucose was increased further for all the compounds in the presence of insulin at both concentrations tested (Fig. 1).

**Alpha-glucosidase inhibitory assay**

The alpha-glucosidase inhibiting activity of curcumin pyrazole derivatives (3a-3e) at different concentrations (50-200 µmol/l) was studied, and the results are depicted in the Fig. 2.

The enzyme alpha-glucosidase inhibitory effect was found to be maximum for the compound 3b, and it showed 17.39±0.64, 38.78±0.43,

**Table 1: Glucose uptake assay using the porcine diaphragm**

| Sample description | Glucose uptake at 400 µmol/l (mg/g/30 minutes) | | Glucose uptake at 800 µmol/l (mg/g/30 minutes) | |
|---|---|---|---|---|
| | Without insulin | With insulin | Without insulin | With insulin |
| Curcumin | 24.12±0.32 | 27.71±0.41 | 29.81±0.62 | 31.73±0.29 |
| 3a | 22.99±0.27 | 26.38±0.36 | 28.46±0.44 | 31.42±0.41 |
| 3b | 23.87±0.43 | 27.75±0.29 | 28.98±0.37 | 31.39±0.35 |
| 3c | 21.82±0.39 | 23.77±0.33 | 25.50±0.28 | 28.30±0.27 |
| 3d | 21.08±0.47 | 23.54±0.47 | 25.53±0.34 | 28.24±0.33 |
| 3e | 22.86±0.36 | 24.89±0.52 | 26.54±0.46 | 29.44±0.53 |
| Control | 20.56±0.54 | 23.35±0.38 | 20.56±0.54 | 23.35±0.38 |

Data represents mean±SEM (n=3), SEM: Standard error mean

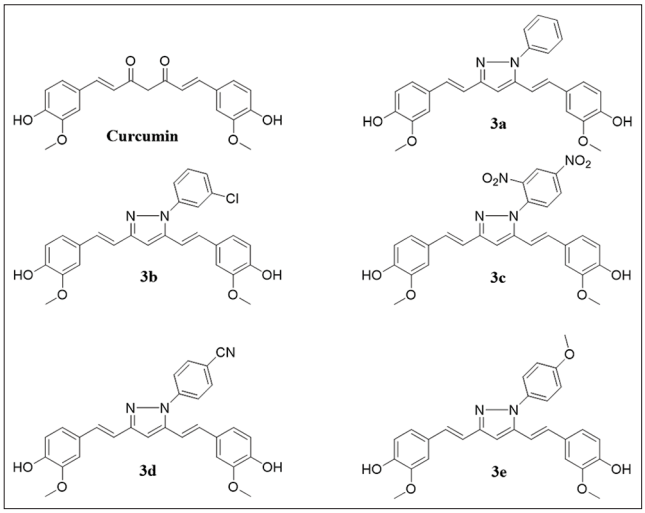


**Fig. 1: Structure of curcumin and curcumin pyrazole derivatives (3a-3e)**

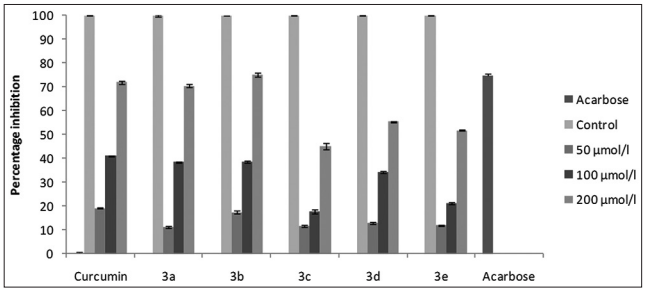


**Fig. 2: Alpha-glucosidase inhibitory effects of pyrazole derivatives of curcumin (50-200 µmol/l) and acarbose showing percentage inhibition. Values are expressed as mean±standard error mean (n=3)**

and 75.29±0.87% inhibition at 50, 100, and 200 µmol/l concentrations, respectively. It was comparable to the effect of curcumin that showed 19.18±0.33, 41.06±0.27, and 71.98±0.64% of inhibition of the enzyme activity at the respective concentrations of 50, 100, and 200 µmol/l. Compound 3a also exhibited considerable inhibitory activity, and it was found to be 11.11±0.57, 38.50±0.26, and 70.53±0.66% at 50, 100, and 200 µmol/l concentrations, respectively. The other compounds in the series showed moderate inhibitory activity, and it was found to be 45.06±1.35, 55.34±0.15, and 51.82±0.17% at 200 µmol/l concentrations of compounds 3c, 3d, and 3e, respectively. Acarbose exhibited 74.81±0.55% inhibition of the enzyme activity at 100 µmol/l concentration.

**Sucrase inhibitory assay**

The sucrase inhibition studies of curcumin pyrazole derivatives (3a-3e) at different concentrations (50-200 µmol/l) were performed, and the results are shown in the Fig. 3.

Compound 3b exhibited a prominent inhibitory activity in the series of curcumin pyrazoles tested. It showed 34.26±0.21, 46.43±0.23, and 75.82±0.17% inhibition at 50, 100, and 200 µmol/l concentrations, respectively. Curcumin showed the utmost activity and the inhibitory activity was found to be 32.17±0.58, 48.52±0.26, and 81.04±0.65% at 50, 100, and 200 µmol/l concentrations, respectively. Other compounds in the series exhibited considerable sucrase inhibition and were found to be 66.08±0.15, 50.43±0.64, 57.73±0.86, and 47.47±0.65% for 3a, 3c, 3d, and 3e, respectively, at 200 µmol/l concentration. Acarbose showed 79.30±0.23% inhibition at 100 µmol/l concentration.

**Alpha-amylase inhibitory assay**

The alpha-amylase inhibitory potential of curcumin pyrazole derivatives (3a-3e) at different concentrations (50-200 µmol/l) was evaluated, and the results are shown in the Fig. 4. Compound 3a and 3b exhibited significant inhibitory activity against amylase and was comparable with that of curcumin. Compound 3a showed 37.35±0.15, 53.87±0.57, and 71.98±0.41% inhibition at 50, 100, and 200 µmol/l concentrations, respectively. Compound 3b was found to possess pronounced inhibitory activity with 45.68±0.17, 55.74±0.34, and 80.02±0.23% inhibition at 50, 100, and 200 µmol/l concentrations, respectively. The curcumin exhibited maximum inhibitory activity and was 43.96±0.97, 57.47±0.52, and 84.33±0.74% at the respective concentrations of 50, 100, and 200 µmol/l. Compounds 3c, 3d, and 3e also showed substantial inhibition at 200 µmol/l and it was found to be 59.05±0.27, 65.08±0.26, and 56.60±0.43% for each compound, respectively. Acarbose showed 80.02±0.31% inhibition at 100 µmol/l concentration.

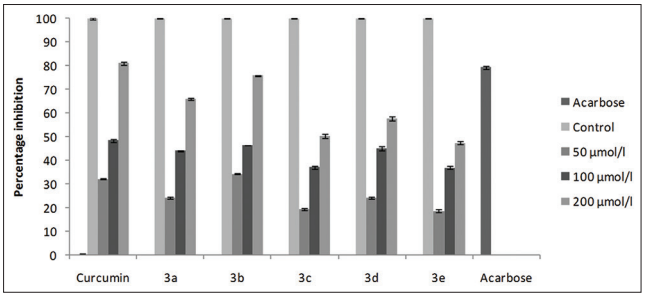


**Fig. 3: Sucrase inhibitory effects of pyrazole derivatives of curcumin (50-200 µmol/l) and acarbose showing percentage inhibition. Values are expressed as mean±standard error mean (n=3)**

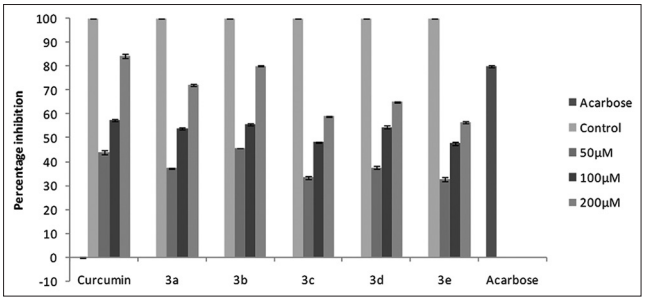


**Fig. 4: Alpha-amylase inhibitory effects of pyrazole derivatives of curcumin (50-200 µmol/l) and acarbose showing percentage inhibition. Values are expressed as mean±standard error mean (n=3)**

## DISCUSSION

Retarding the absorption of glucose is one of the promising approaches for treating diabetes. This can be done by inhibiting various carbohydrate hydrolyzing enzymes in the digestive tract [24]. The above enzymes studied for their inhibition play a vital role in converting polysaccharides into simple sugars. The simple sugars formed, mainly the glucose was absorbed through the intestinal epithelial cells by sodium-dependent, carrier-mediated active transport [25]. The components that are able to prevent at least one of the above events can be an effective hypoglycemic agent. Inhibition of the enzymes, alpha-glucosidase, and alpha-amylase decreases the hydrolysis of starch into simple sugars. Sucrase cleaves sucrose into glucose and fructose, the inhibition of which control the conversion of disaccharides into simple sugars.

In the present study, the curcumin pyrazole series (3a-3e) exhibited hypoglycemic activities in the *in-vitro* models studied. The diaphragm is an important striated muscle tissue, which utilizes glucose from the blood and thus decreases blood glucose. All the compounds in the series shown to possess increased absorption of glucose and the action of insulin were also enhanced in the presence of curcumin pyrazoles. Among the curcumin pyrazole derivatives tested, compound 3b and 3a were found to be more effective in increasing the absorption of glucose. The enzyme alpha-amylase was inhibited by the curcumin pyrazole derivatives (3a-3e), out of which 3b was found to be more potent as that of curcumin. Compound 3a was also found to be an effective inhibitor of alpha-amylase activity. The activity of intestinal alpha-glucosidase and sucrase was also shown to inhibited by the series of compounds (3a-3e) tested. Compounds 3a and 3b exhibited significant inhibition, and the compound 3b was found to be a prominent inhibitor of the carbohydrate hydrolyzing enzymes tested.

In the previous studies, an ethanolic extract of *Alpinia calcarata* was found to enhance the uptake of glucose in rat hemidiaphragm [26]. Similarly, *Momordica charantia* fruit extract has been shown to increase the glucose uptake in the hemidiaphragm of the diabetic rats [27]. One of the flavanoids, luteolin, was found to be a potent inhibitor of alpha-glucosidase from yeast and the activity of the enzyme was inhibited by 36% at 0.5 mg/ml [28]. The bark extracts of *Pinus densiflora* exhibited an effective inhibition against alpha-glucosidase and alpha-amylase enzymes of different origins [29]. Two bromophenols, 2,4,6 tribromophenol and 2,4 dibromophenol purified from red alga *Grateloupia elliptica* showed $IC_{50}$ values of 60.4 and 110.3 µmol/l against alpha-glucosidase from yeast, respectively, and $IC_{50}$ values of 130.3 and 230.3 µmol/l against alpha-glucosidase from *Bacillus stearothermophilus,* respectively. These compounds also showed to inhibit rat intestinal sucrase and rat intestinal maltase [30]. Bisdemethoxycurcumin, a natural derivative obtained from the rhizome of *Curcuma longa* inhibited human and porcine pancreas alpha-amylase with the $IC_{50}$ values 0.025 and 0.026 mmol/l, respectively [31]. The potent inhibitory activity of the curcumin pyrazole series (3a-3e) can be due to the polyphenolic and pyrazole moiety present in the compounds of the series. Moreover, the multiple actions and minimal side effects of the curcumin could be a key factor in the superiority of curcumin analogs over the other drugs which are under investigation [32]. The higher inhibitory efficiency of the compound 3b can be attributed to the electron donating capacity of the chlorine atom. The structure-activity relation needs to be assessed in the future studies.

## CONCLUSION

Curcumin pyrazole derivatives (3a-3e) were assessed for antidiabetic effects of the compounds by the inhibition studies of carbohydrate hydrolyzing enzymes, including alpha-amylase, rat intestinal alpha-glucosidase, and sucrase in different *in-vitro* assays. The hypoglycemic effect of curcumin pyrazole derivatives (3a-3e) was also assessed by the glucose absorption assay using the porcine diaphragm. Though all the compounds investigated showed hypoglycemic activity by inhibiting the enzymes studied, the inhibition was not significant when compared to the inhibitory activity of 3a and 3b. The compounds 3a and 3b comparatively increased the uptake of glucose through the porcine diaphragm than the other compounds in the series. The report suggested that the curcumin pyrazole derivatives (3a-3e) have antidiabetic activities *in-vitro*. Compound 3a and 3b were found to possess a greater percentage of inhibitory activity, out of which compound 3b, 3c, 3d and 3e was found to be more potent. From the findings, we can conclude that the synthetic analog of curcumin, compound 3a and compound 3b could be promising antidiabetic agents and can be selected for further *in-vitro* and *in vivo* investigations.

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