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Synthesis and Anti Diabetic Activity of Curcumin

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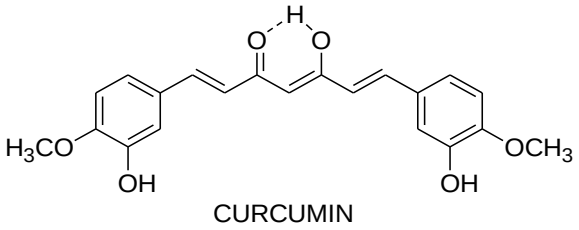
INTRODUCTION

Scientific NAMES: *Curcuma Longa, C. Domestica*
Common names: turmeric, curcuma, Indian saffron.

Botany: The plant curcuma longa of family zingiberaceae is a perennial herb widely cultivated in the tropical region of Asia. Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are polyphenols and are responsible for the yellow color of turmeric. Curcumin can exist in at least two tautomeric forms, keto, and enol. The enol form is more energetically stable in the solid phase and in solution.

Curcumin is one such medicine. Turmeric, derived from the plant *Curcuma longa*, is a gold-colored spice commonly used in the Indian subcontinent, not only for health care but also for the preservation of food and as a yellow dye for textiles. Curcumin, which gives the yellow color to turmeric and its structure as diferuloylmethane. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities and thus has a potential against various malignant diseases, diabetes, allergies, arthritis, Alzheimer's disease, and other chronic illnesses. These effects are mediated through the regulation of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes.

STRUCTURE



CURCUMIN

Fig 1.1: IUPAC NAME: (EE) 1, 7-Bis (4-hydroxy-3 methoxyphenyl)-1, 6 heptadiene-3, 5-dione

CHEMICAL NAME: Diferuloyl methane
MOLECULAR FORMULA: 368.37
CHEMICAL COMPOSITION: C= 68.47%, H=5.47%, O=26.06%
MELTING POINT: 182^0-183 ^{0}C max=435 nm

PROPERTIES OF CURCUMIN

Curcumin has antioxidant, anti-inflammatory, antiviral and antifungal actions. Studies have shown that curcumin is not toxic to humans. Curcumin exerts anti-inflammatory activity by inhibition of a number of different molecules that play an important role in inflammation. Turmeric is effective in reducing post-surgical inflammation. Turmeric helps to prevent atherosclerosis by reducing the formation of blood clumps. Curcumin, its main active constituent, is as powerful and antioxidant as vitamins C, E, and Beta-Carotene, making turmeric usage a consumer choice for cancer prevention, liver protection and premature aging.

Effect of Curcumin on neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases was observed.

SOLUBILITY

Insoluble in water and ether. Soluble in alcohol, glacial acetic acid, gives brownish red colour with alkalies and light yellow colour with acids.

ISPOLATION AND EXTRECTION

The isolation of natural *Curcumin* from the *Curcuma Longa* rhizome is a difficult and costly procedure. No practical way had been found to effect separation of Curcumin itself from two related demethoxy compounds with which it is found in nature. This difficulty of separation has led to several attempts to synthesize the compound, the most important of which had been aldol condensation of vanillin (3-methoxy-4-hydroxybenzaldehyde) and 2, 4-pentanedione. However, the yields of product from these syntheses have therefore been very low, in large part because of the difficult and complicated procedures required for isolation and purification of the product.

MATERIALS AND METHODS

1. PRIMARY REACTANTS

The curcumin derivatives are generally synthesized by derivatization, starting from curcumin. For example, the phenolic hydroxy group may be acylated, alkylated, glycosylated, and amino acylated.

The primary reactants for the process of the reaction are 2, 4-diketones and aromatic aldehydes. The diketones suitable for use in the process of the reaction are those corresponding to the structural formula $H_2RC\text{-}CO\text{-}CH_2\text{-}CO\text{-}CRH_2$, in which the R groups are independently selected from H and $C_{1\text{-}12}$ hydrocarbyl groups selected from alkyl, aryl, aralkyl, alkaryl groups and mixtures thereof. Acetylacetone, i.e. 2, 4-pentanedione, is preferred for use in the invention.

Other suitable diketones include 3-substituted-2,4-pentanediones, $RCH(COCH_3)_2$, where R is $CH_2{=}CHCH_2$, $CH_3(CH_2)_3$, $(CH_3)_2CH$, $C_2H_5CO_2CH_2$, $C_2H_5O_2C(CH_2)_2$, $HO_2C(CH_2)_2$.

2. **SOLVENT**

Suitable solvents for use in the reaction include highly polar, aprotic solvents, especially organic amides such as N, N-dimethylacetamide, N, N-dimethylformamide, N-methylpyrrolidinone, N-formylpyrrolidine and the like. Even though it is highly polar and aprotic, dimethyl sulfoxide is not suitable for use in the reaction because it forms a tarry mass from which the curcumin-related product is extremely difficult to separate except with excessive losses in yield.

3. CATALYST

Suitable catalysts for use in the process of the reaction are primary and secondary amines such as morpholine, n-Butylamine, ethanolamine, and diallylamine. Tertiary amines such as triethylamine are technically operable for use in the process, but are less effective catalysts and require excessive reaction times to obtain suitable yields. They are therefore not preferred for use in the reaction.

4. WATER SCAVENGER

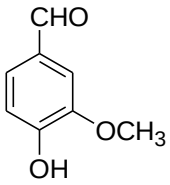
Water in the reaction systems, irrespective of its source, can react with the diketone complex in the reaction mixture and thus substantially reduce the yield of curcumin. To accomplish this, it is desirable to incorporate a scavenger into the reaction system which will bind with the water and prevent its reaction with the diketone complex. Suitable scavengers for this purpose have been found to be $C_{1\text{-}5}$ alkyl borates and $C_{1\text{-}5}$ alkyl phosphates and mixtures thereof.

4.1 CHEMICAL SYNTHESIS OF CURCUMIN (DIFERULOYL METHANE)

REQUIREMENTS

1. Acetyl acetone (2,4 pentanedione) - 1.0 gm (0.01 mol)
2. Boric anhydride (B_2O_3) - 0.05 gm (0.007 mol)
3. Ethyl acetate - 10 ml
4. Tributyl borate - 9.20 gm (0.02 mol)
5. n-butyl amine - 1.10 gm (0.015 mol)
6. Vanillin - 3.04 gm (0.02 mol)
7. Sodium sulphate (anhydrous)
8. Dilute Hcl: 10 ml (8.5 ml H_2O + 1.5 ml conc. HCL)

The amount of vanillin (4-hydroxy-3- methoxy benzaldehyde) to be used was calculated as below from its structure as below:-



$= C_8H_8O_3 = 152$ (M.Wt.)

152 * 0.02 = 3.04 gm.

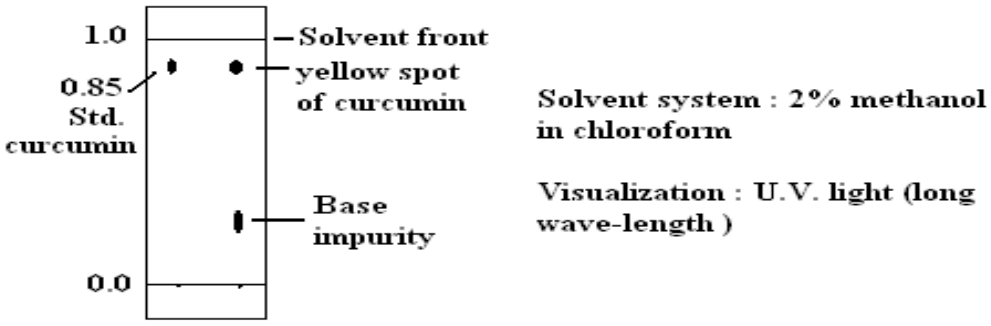
Fig 4.2 (4-hydroxy-3- methoxy benzaldehyde)

PROCEDURE AND OBSERVATION

In a 100 ml three necked round bottom flask, placed on magnetic stirrer was charged with 1.0 ml of acetyl acetone, 0.068 gm of boric anhydride and 10 ml of ethyl acetate solution which was then stirred for a duration of 60 minutes. 9.2 ml of tributyl borate and 3.04 gm of vanillin were added and stirring continued at room temperature. At almost 45 minutes later 1.1 gm of n-butyl amine dissolved in 10 ml of ethyl acetate solution was added dropwise from an addition funnel, at this point colour change was clearly visible. The reaction required at least 8 hrs of reaction (left overnight).

The next step involved acid hydrolysis of the reaction mixture and for that 1.5 ml of conc. HCL was diluted (dissolved) using 8.5 ml of distilled water then added dropwise over a period of 30 minutes with constant stirring. The next step was placing the system on an oil bath and heating the contents upto 60°C for 2 1\2 hrs with constant stirring.

To monitor the progress, TLC analysis was done on the crude product. This involved taking some very little sample of the compound in a test tube, dissolved in methanol and spotted against a standard (dissolved in methanol) on precoated TLC plate.



(TLC of the crude product)

R_f = distance travelled by spot/distance travelled by solvent.
R_f = 0.85/1 = 0.85 curcumin
R_f = 0.15 - base impurity.

From TLC result it was clear that the product had been formed and now the reaction mixture could be worked up to extract the product.

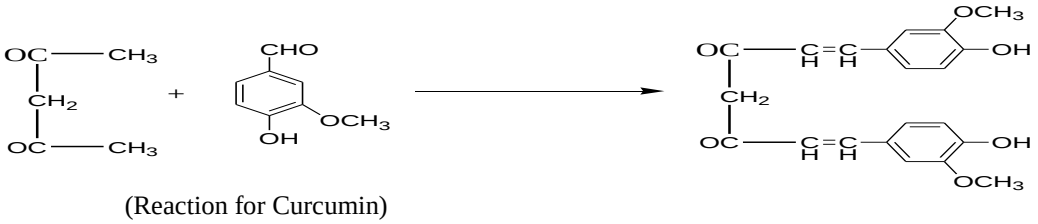
OBSERVATION MADE DURING WORKUP

A problem encountered during the workup period was the difficulty in separating the organic and aqueous layer. This happened normally due to the formation of emulsion (usually called a muck) which makes it hard for separation to occur. I learned how to handle the problem by simply adding a pinch of common salt (sodium chloride) or brine solution which breaks it up facilitating the separation.

DISCUSSION OF THE REACTION SCHEME AND MECXHANISM INVOLVED IN THE SYNTHESIS OF CURCUMIN THEORETICALLY:-

Vanillin + acetyl acetone ---(1) ethyl acetate, (2) boric anhydride, (3) tributyl borate, (4) n- butylamine---> curcumin

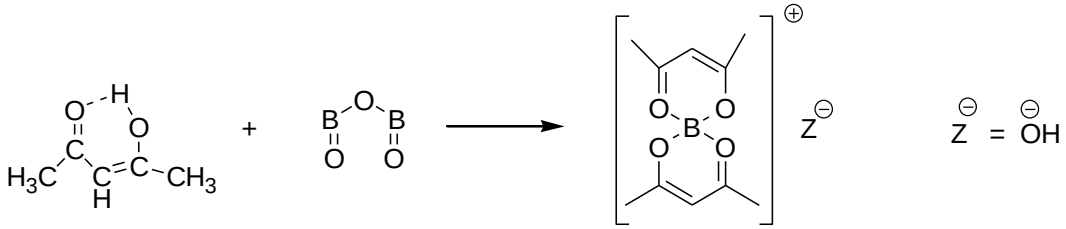
EQUATION:



(Reaction for Curcumin)

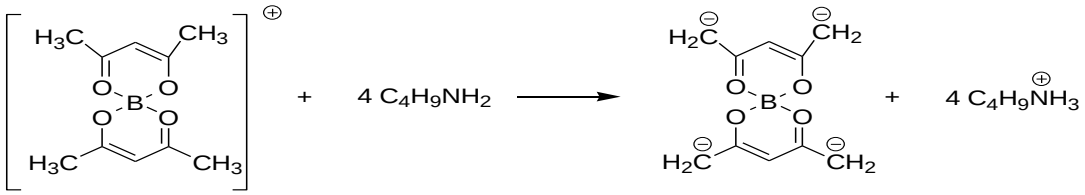
MECHANISM OF THE REACTION

a) At first, there is a boron complex formation on charging acetyl acetone with a boric anhydride in a medium of ethyl acetate.

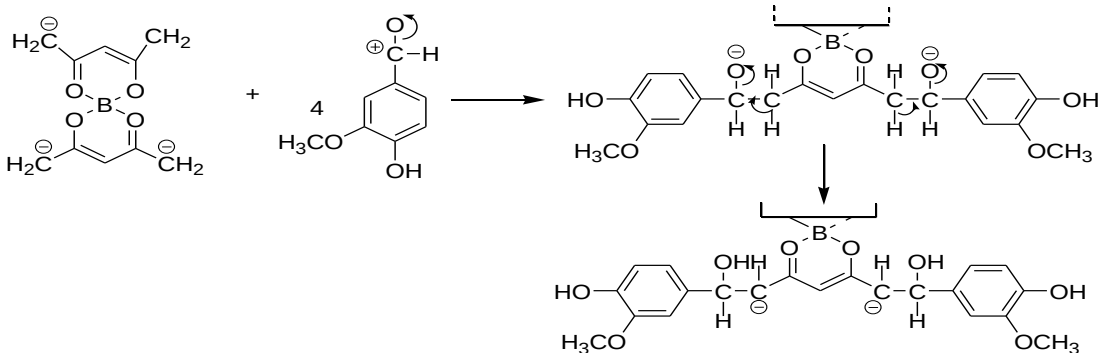


b) The complex formed react 4 molecules of n- butyl amine, which then extracts 4 protons from the methyl group of the complex generating a carbanion which becomes an intermediate:

c)



d) The intermediate generated above reacts with vanillin (aldehyde) molecule to generate another intermediate



e) The intermediate will then remove a proton from $C_4H_9NH_3^+$ and then on hydrolysis using an acid (HCL) at 60°C the complex breaks down releasing the product

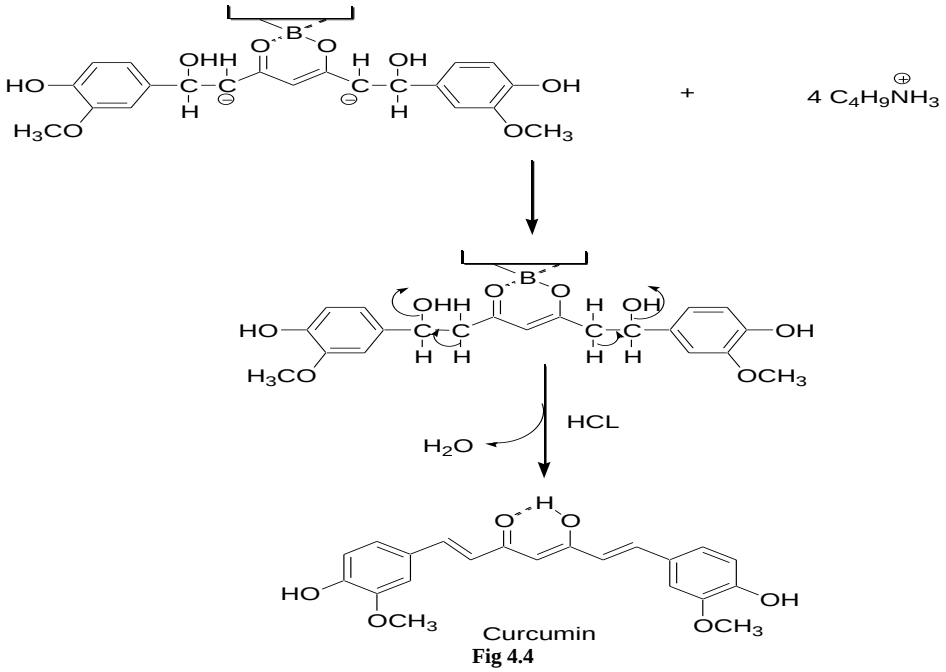


Fig 4.4

ANTI-DIABETIC ACTIVITY

Experimental Animals and Research Protocol Approval

Male wistar rats (150–180g) were taken. Animals were maintained in an air-conditioned room at 22 ± 2^0 C and relative humidity of 45–55% under a 12h light: 12 h dark cycle. The animals had free access to standard food pellets and water was available ad libitum. The experimental protocol was approved by the Central Drug Research Institute Lucknow and constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Induction of Experimental Diabetes and Determination of the Serum Glucose Level

Rats were deprived of food for 16 hours (fasted state) before the induction diabetes. Diabetes was induced in male wistar rats by a single intraperitoneal injection of aqueous alloxan monohydrate (80 mg/kg) solution and the serum glucose level determined by the glucose oxidase peroxidase method. The rats showing a serum glucose level above 300 mg/dl (diabetic state) were selected for this study. Blood samples from the experimental rats were collected by retro-orbital plexus technique using heparinised capillary glass tubes. The collected blood samples were centrifuged at a speed of 7000 rpm for 15 min to get serum. Ten microliters of serum and 1ml of working reagent (GOD/POD) were mixed and incubated for 15 min at 37^0 C. The UV–VIS spectrophotometer (Elico SL 120) reading was adjusted to 0 by measuring the absorbance of blank (distilled water). The absorbance of the sample (As) and standard Astd provided by the manufacturer were measured against blank at 505 nm.
Glucose was estimated by using the formula:

$$\text{Glucose (mg/dl)} = \frac{\text{As}}{\text{Astd}} \times 100$$

Whereas, As = sample reading; Astd =standard reading.

Effect of Curcumin Derivatives on Serum Glucose Levels in Alloxan Induced Diabetic Mice:

The selected rats were divided into 10 groups (n =3), viz

1. Group I— Alloxan (80 mg/kg, Diabetic control),
2. Group II— Alloxan + Glibenclamide (10mg/kg),
3. Group III— Alloxan + Vehicle (CMC 1%, 0.5ml/rat),
4. Group IV— Alloxan + Curcumin (100mg/kg),
5. Group V— Alloxan + Compound (**1**) (100 mg/kg),
6. Group VI— Alloxan + Compound (**2**) (100 mg/kg)
7. Group VII— Alloxan + Compound (**3**)I(100 mg/kg)
8. Group VIII— Alloxan + Compound (**4**) (100mg/kg),
9. Group IX— Alloxan + Compound (**5**) (100mg/kg),
10. Group X — Alloxan + Compound (**6**) (100mg/kg).
11. Group XI — Alloxan + Compound **(7)** (100mg/kg).
12. Group X II— Alloxan + Compound **(8)** (100mg/kg).
13. Group XIII — Alloxan + Compound **(9)** (100mg/kg).
14. Group XIV — Alloxan + Compound (**10**) (100mg/kg).

All compounds were given orally while alloxan was given intraperitoneally. Rats fasted overnight before the commencement of the study. The study involves the determination of serum glucose levels at 0, 1, 2, 4, 6 and 8 hours after administration of all compounds.

5.2 STATISTICAL ANALYSIS

Data were expressed as Mean± S.E.M. and statistical analysis was carried out by one-way ANOVA with Student- Newmann-Keuls test performed using GraphPad Prism windows 5.02 for Windows Vista™ BASIC, GraphPad Software, San Diego, California, USA, www.graphpad.com. *p* value was considered significant when <0.05.

RESULT AND DISCISSION

6.1 SYNTHESIS

Curcuminoids which were synthesized were been given for spectral studies and on basis of their spectra (I.R., NMR, Mass) and melting point it was confirmed that the synthesized curcuminoids were pure and for few curcuminoids column chromatography was been done in the surge to obtain pure compounds.

Compound 1- "**Curcumin**"
m.p. - 180-181^{0}C
% yield - 47%
Compound 2- "**Trimethoxy Curcuminoid**"
m.p.- 298-300^{0}C
% yield- 57 %
Compound 3- **"Piperonal curcuminoid**"
m.p.- 193-195^{0}C
% yield- 43%
Compound 4- "**p-hydroxy Curcuminoid**"
m.p.- 221-223^{0}C
% yield- 52%
Compound 5- "**p-Chloro curcuminoid**"
m.p.- 158-160^{0}C
% yield- 29%
Compound 6-"**m-Nitro curcuminoid**"
m.p.- 144-146^{0}C
% yield- 19%
Compound 7- **"p-Methoxy curcuminoid**"
m.p.- 208-210^{0}C
% yield- 70%
Compound 8-"**3,4-Dimethoxy curcuminoid**"
m.p.- 130-131^{0}C
% yield- 55%
Compound 9-"**p-Fluoro curcuminoid**"
m.p.- 151-153^{0}C
% yield- 27%
Compound 10-"**p-Methoxy curcuminoid**"
m.p.- 166-167^{0}C
% yield- 37%
Compound 11-"**Dihydroxy curcuminoid**"
m.p.- 304-306^{0}C
% yield- 40%

6.2 ANTI DIABETIC ACTIVITY

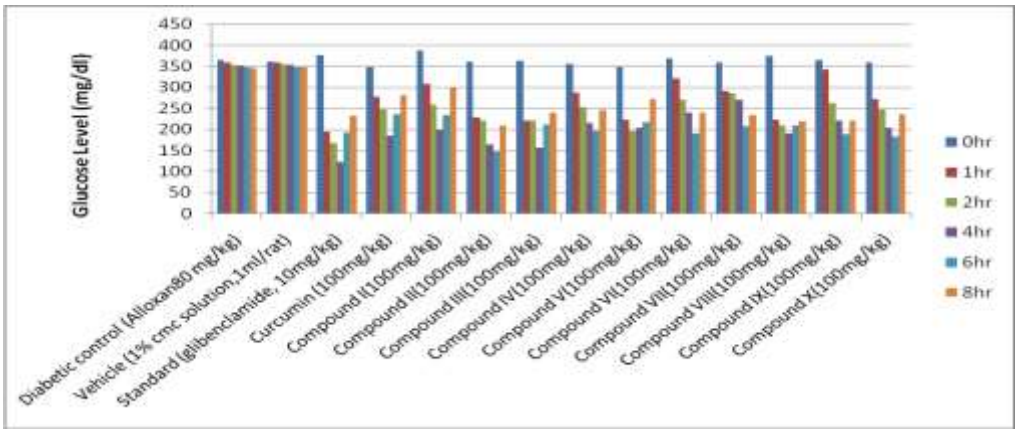
Alloxan injection produced hyperglycaemia in all animals. The single dose administration of the glibenclamide, curcumin and the synthesized compounds to diabetic animals significantly reduced the glucose serum glucose levels at 1, 2, and 4 hours. The standard drug glibenclamide produced maximum activity within 4 hours (reduced initial serum glucose levels up to 67 %).
The data is shown in **Table 1** and **Fig 6.1** represents the effect of drugs on serum glucose levels in diabetic rat.

Table 1: Effect of Drugs on Serum Glucose Levels in Diabetic Rats [a, b]

Group	**Mean ± SEM glucose level (mg/dl)**					
	0hr	**1hr**	**2hr**	**4hr**	**6hr**	**8hr**
Diabetic control (Alloxan80 mg/kg)	364.7±6.766	358.7±7.688	354±6.245	352±4.41	347.1±3.606	346.7 ±4.41
Vehicle (1% cmc solution,1ml/rat)	362±5.13	358.7±4.485	356.3±5.667	352.7±6.36	350±7.55	348.3 ±4.41
Standard (glibenclamide, 10mg/kg)	376±5.859	195.3±2.906	169±2.646	122.3±2.404	192.7±2.33	233.3 ±4.055
Curcumin (100mg/kg)	347.3±4.4485	278±3.606	248.3±4.333	184.7±4.256	236.7±3.528	281.7 ±3.844
Compound I(100mg/kg)	386.7±1.202	307.7±2.186	258.7±2.33	198.3±2.028	235±2.887	299±8 .185
Compound II(100mg/kg)	361.7±11.61	229.3±6.009	221.3±8.090	165.3±5.044	147.3±5.044	209.7 ±6.33
Compound III(100mg/kg)	363.7±12.14	222±7.234	220.7±6.064	157±5.859	211.3±7.055	241±8 .327

Compound IV(100mg/kg)	355.3±5.783	287.3±8.950	254.3±4.631	214.7±5.044	196.3±4.095	246.7 ±2.96 3
Compound V(100mg/kg)	349.3±3.383	223.3±4.177	196.3±2.028	205±2.883	216.7±1.764	272±2 .517
Compound VI(100mg/kg)	369.3±8.09	322.36±6.74	270.7±7.05	239.7±8.373	191.3±4.667	239.7 ±5.36 4
Compound VII(100mg/kg)	359.5±4.309	291.4±7.36	285.4±5.771	271±4.302	207.2±3.497	234.6 ±7.23 8
Compound VIII(100mg/kg)	373.7±10.14	224±4.531	209.7±5.362	191±3.958	209.3±8.157	219±6 .352
Compound IX(100mg/kg)	365.7±9.034	342.6±7.121	262.2±9.021	221.3±5.735	189.2±5.463	221.3 ±7.65 4
Compound X(100mg/kg)	358.7±6.756	271.5±6.764	247.4±7.564	203.4±8.146	183.9±10.75	236.5 ±4.76 5

Figure 2: Effect of Drugs on Serum Glucose Levels in Diabetic Rat



Curcumin, Compound I and Compound V produced maximum activity within four hours only and reduced initial serum glucose level upto 45%, 48%, 56% and 41% respectively. The onset of action of all compound was observed after 1 hour.

DISCUSSION

A problem encountered during the experimental work was the difficulty in separating the organic and aqueous layer. This happened normally due to the formation of emulsion (usually called a muck) which makes it hard to separate. The problem was solved by means of adding a pinch of common salt (sodium chloride) or brine solution which facilitates the separation ketones.

CONCLUSION

The objective mentioned was achieved by synthesizing different curcuminoids and structure were confirmed by means of NMR, FT-IR and Mass spectra. Also measured the melting point for all compounds and compared with the standard. All the compounds synthesized were well crystalline.

It can be concluded that all the synthesiszed curcumin structural analogue possessed anti-daibetic activity comparable to curcumin in the Alloxan induced rat diabetic model among the 10 compound synthesized, compound 1, compound 2, compound 3, compound 5 shows highly significant activity then curcumin and comparable to standard drug glibenclamide.

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