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SOLUBILITY ENHANCEMENT OF CO-CRYSTAL BASED SOLID DOSAGE FORM

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ABSTRACT

Pharmaceutical co-crystallization has allured a lot of attention by means of altering the physicochemical properties of Active Pharmaceutical Ingredient (API) such as solubility, stability and bioavailability. Crystal engineering of curcumin can produce novel compounds such as pharmaceutical co-crystals. The present investigation involves in formulation and solubility enhancement of a co-crystal based solid dosage form consisting of a stoichiometric amount of parent drug curcumin with a pharmaceutically acceptable co-former resorcinol. Firstly co-crystals are prepared through liquid assisted grinding method, crystal confirmatory tests done by FT-IR, DSC and PXRD. Delivery of an API is generally preferred in a solid dosage form.

KEYWORDS: Crystal engineering, Curcumin, FT-IR, DSC and PXRD.

INTRODUCTION

Co-crystals are most commonly thought of as structural homogeneous crystalline materials that contain two or more neutral building blocks that are present in definite stoichiometric amounts and are obtained through the establishment of strong hydrogen bonds and other non-covalent interactions such as halogen bonds, π-π and columbic interactions [1]. The emerging field of co-crystal allows modifying the composition of matter and physicochemical properties of a molecule with or without breaking or forming a covalent bond. An attractive subset of cocrystals is pharmaceutical co-crystals. The purpose of this investigation is to explore the possibility of employing "Co crystallization" in developing an aqueous soluble co crystal'. This technique may provide the synergistic enhancement in aqueous solubility of poorly water-soluble drugs.

The growing public interest in traditional medicine, particularly plants-based medicine, has led to extensive research on the potentials of natural origin substances. Hundreds of studies were conducted to investigate the effects of natural origin compounds on human health and prevention and treatment of chronic diseases [2]. Among studied compounds, polyphenols appear as one of the most promising groups. In plants, polyphenols are important for growth and protection against pathogens. The utility of curcumin is limited by its, lack of water solubility, and relatively low *in vivo* bioavailability.

Present investigation deals with the approach of enhancement of solubility of curcumin by using a coformer resorcinol utilizing supramolecular technique. Moreover the study also determines the effect of solvent and the method influence the solubility and dissolution competence of curcumin.

MATERIALS AND METHODS

Curcumin was procured from ITC Guntur; Resorcinol was purchased from Himedia Laboratories Pvt. Ltd, Mumbai. All the other chemicals and excipients used were of analytical grade.

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Formulation of co-crystals

Curcumin and resorcinol in 1:1 ratio were grinded in a mortar and pestle using small quantity of ethanol by liquid assisted grinding method. The crystals formed were collected separately and preserved.

Characterisation of co-crystals

The pure drug, excipient and the co-crystals obtained from neat grinding and solvent evaporation methods were subjected to FT-IR, DSC, SEM, PXRD studies.

Infrared spectroscopy (FTIR)

IR spectroscopy was conducted using a FTIR Spectrophotometer (Thermo-IR 200) and Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. The spectrum of curcumin, resorcinol and co-crystals was recorded in the wavelength region of 4000–400 cm−1 [3].

Differential scanning calorimeter (DSC)

Thermal analysis of curcumin, resorcinol, and co-crystals were recorded on a DSC (NETZSCH DSC 204). The temperature axis and cell constant of DSC were previously calibrated with indium. A heating rate of 100C/min was employed with nitrogen pursing. Powder samples (15 -30 mg) was weighed into an aluminium pan and analyzed as sealed with pin holes and an empty aluminium pan was used as reference [4].

Scanning electron microscopy (SEM)

The surface characteristics of resorcinol, and co-crystals were studied by SEM (ZEISS Electron Microscope, EVO MA 15). The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode [5].

Powder X-Ray Diffraction (PXRD)

The PXRD were undertaken to investigate the crystalline nature of resorcinol, and co-crystals. The study was carried out using X-Ray Diffractometer using Cu kα radiation. The tube operated at 45 kV, 9mA and data was collected over an angular range from 0 to 500 2θ of the diffraction angle in continuous scan mode using a step size of 0.050 2θ and a time of 0.1 s [6].

Formulation of co-crystals into tablets

Pharmaceutical solids occur in different crystal forms. Different forms of same crystal show different behaviour during compression and in dissolution studies. So, the right crystal form should be selected for direct compression with suitable excipients. Direct compression reduces production cost, gives better product stability and faster dissolution of drug.

***IN-VIVO* PHARMACOLOGICAL ACTIVITY**

Animals used

Healthy adult wistar rats (180-200gm) were maintained in a well ventilated room with 12:12 hour light or dark cycle in polypropylene cages. The animals were fed with standard pellet fed and water was given ad libitum. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

ANTIULCER ACTIVITY

Ethanol induced gastric ulcer

Animals were randomly divided into four groups each of 6 rats. Group I treated with 1% w/v CMC (10 ml/kg p.o), Group II treated with Pure Curcumin (100mg/kg p.o) Group III treated with prepared crystals (100mg/kg p.o) respectively for 5 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 5th day, Gastric ulcers were induced with ethanol at a dose of 8ml/kg administered to all groups by orally [7]. The animals were anaesthetized 6 h with ether and stomachs were incised along the greater curvature and the ulcer index for each rat was taken as the mean ulcer score.

Measurement of ulcer index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%I) was calculated as described by Nguelefack et al. [8] using the following formula:

$$\%I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

Histopathological studies

The freshly excised stomachs were washed with saline and preserved in 10% formaldehyde solution for histopathological studies. The sections of stomachs stained with hematoxylin and eosin, were assessed for histopathological changes such as congestion, edema, hemorrhage and necrosis [9]. The microscopic slides were photographed.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way

analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS AND DISCUSSION

Solubility Studies

The solubility studies of pure compounds and co-crystal were determined using a 24-hour shake flask method (used previously for many compounds). To 1ml of the solvent excess amount of drug is added and it is kept for stirring for 24 hr in orbital shaker. After 24 hrs the samples with sufficient dilutions was analysed spectrophotometrically at 431 nm. The solvent used are P^H 1.2, the solubility studies have been performed for the pure drug and prepared crystals. In pH 1.2 buffer the pure drug showed a solubility of 0.08 mg/ml, while liquid assisted grinding methods showed a solubility of 0.157 mg/ml respectively

Infrared spectroscopy (FTIR)

FT-IR results show that the crystals prepared from liquid assisted grinding method has change in their peak intensity when compared to pure drug and others. But only a slight change in peak wavelength was identified for pure drug, and crystals prepared from liquid assisted grinding method and the bonds observed are stretching bonds. The values are mentioned in the table 2.

Differential scanning calorimeter (DSC)

DSC experiments were carried out to study the thermal behaviour of the crystal form in relation to the individual components. DSC thermal data are shown in figure. DSC study of curcumin and resorcinol shows endothermic peak at 175.67^0C and 112.02^0C C while DSC study of prepared cocrystal shows sharp endothermic value at 165.75^0C, the sharp endothermic values of crystal form and the individual components agreed with the measured melting range in the melting point determination. The thermal profile of crystal form was distinct, with a different melting transition from that seen with either of the individual components. This indicates the formation of novel crystal phase: crystal form of Curcumin with resorcinol (1:1 molar ratio) figure 2. This single endothermic transition indicates the absence of any unbound or absorbed solvent or water and also demonstrates the stability of the phase until the melting point.

Powder X-Ray Diffraction (PXRD)

Predicted PXRD pattern of crystal form shown in figure19, which was different from its pure drug powder PXRD pattern, this indicates the formation of new multi component crystalline phase. By comparing the above graphs, each graph showing 100% relative intensity at different 2θ ranges, which showing clearly that the formulated crystals having crystal property.

Scanning electron microscopy (SEM) studies

SEM photography of prepared cocrystal shows uniform block or rod like crystals while curcumin is showing different type of crystals and resorcinol showing pellet like crystals when comparing. This indicates the formation of Crystal form. SEM photographs of Curcumin, Resorcinol and prepared crystals are shown in figure 4.

Evaluation of Formulated Tablets

The weight variation test were done for F1 to F6 formulations and found to be 200.1 ± 0.05 to 200.8 ± 0.01. The % deviation is coming within 3% to 5% range for this test accepted % deviation should be 5 % for 200-250 mg tablet. F1 to F6 formulations come within limit and passed the test.

The friability test done for the F1 – F6 formulations was ranged from 0.25 to 0.36 which exactly falls within the limit of standard (0.1 to 0.9 %). The thickness was carried out according to the procedure. The thickness of the tablets ranges from 2.9 ± 0.02 to 3.1 ± 0.03. The hardness for F1 to F5 formulations ranged from 3.8 ± 0.10 to 4.0 ± 0.13, which showing that those are within the limits.

The drug content was determined and the results were given. The drug content was determined by measuring absorbance. The drug content F1 to F6 showed in the range of 96.63± 0.02 to 99.36± 0.03 and F1 showed maximum drug content 99.36± 0.03. Disintegration was determined and the results were given. The disintegration time for F1 – F6 were ranging 4.5± 095 to 5.2± 1.36 min. Uniformity of dispersion was carried out. The residue remaining on the screen was found to be nil hence all the formulations from F1 to F6 passed the test.

IN VITRO STUDIES

Dissolution studies were carried out by USP type II (Basket apparatus) at 50 rpm in PH-1.2 buffer. Temperature was maintained at 37 ±0.5 °C. Aliquots of dissolution media were withdrawn at 5,10,15,30,45,60,90,105 minutes of time intervals and the sample was filtered. Same quantity of fresh media was replaced. The filtered solution was used to determine the drug content. Absorbance was we as used at 431 nm by UV/Visible spectrophotometer.

IN VIVO STUDIES

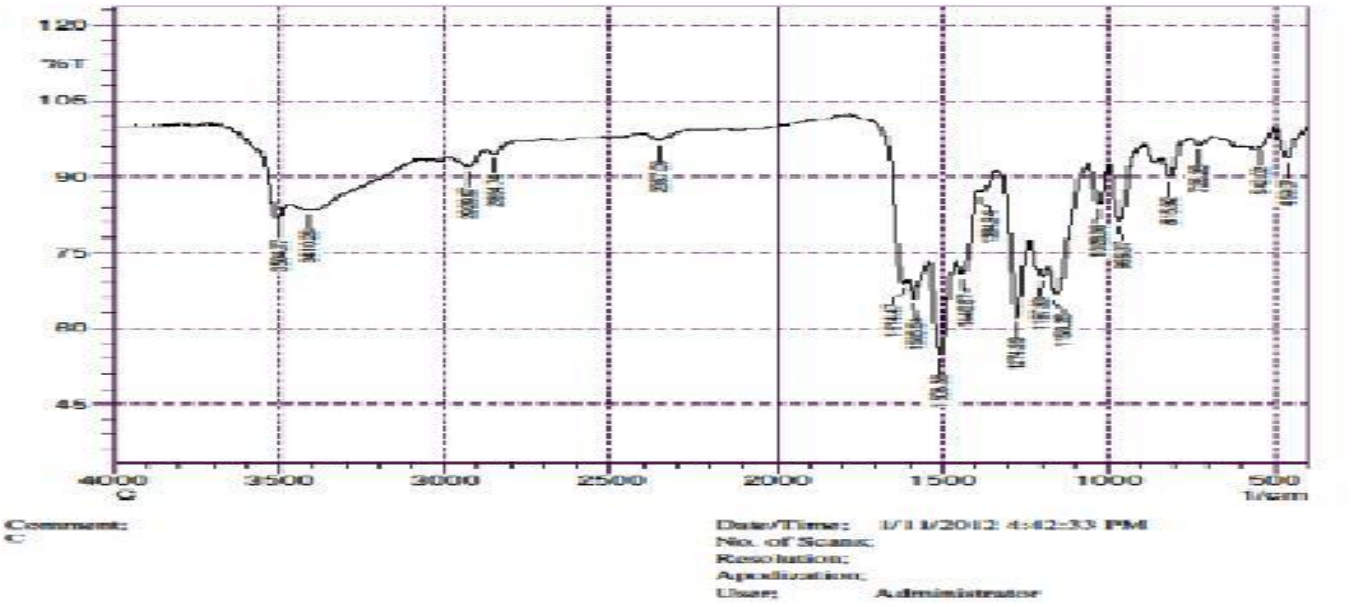
Effect of Co-crystals on ethanol induced gastric ulcer

In this study the prepared crystals were shown significant anti-ulcer activity against ulcers induced by ethanol when compared through Curcumin treated groups. In the ethanol induced ulcer model, prepared crystals at a dose of 10mg/kg body weight showed protective effect of 76.63%, whereas marketed omeprazole showed protection index of 81.47% at a dose of 20mg/kg body weight.

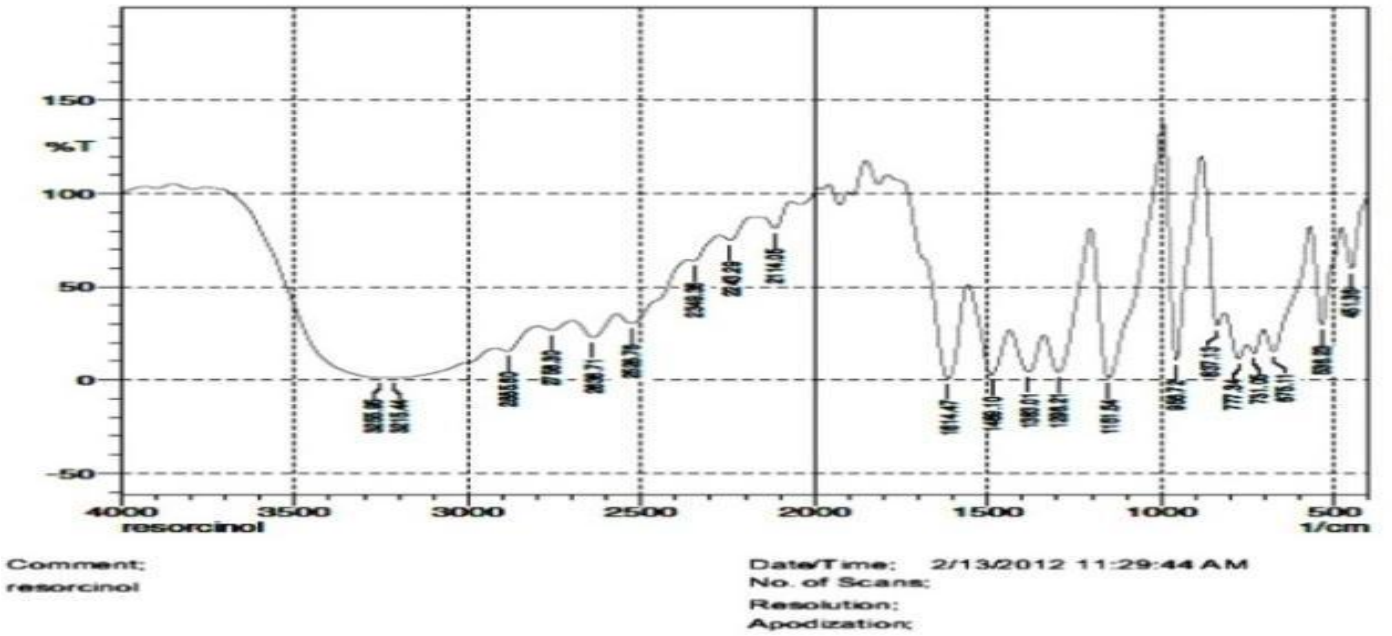
Concerning the histopathological studies, which suggests that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury (disruption of the vascular endothelium) resulting in increased vascular permeability, edema formation and epithelial lifting. Hence this study confirmed that prepared crystals showed significant inhibition in ethanol induced gastric lesions.

Figure 1. Comparative FTIR spectra of curcumin (A), resorcinol (B) and prepared crystals (C)

A



B



C

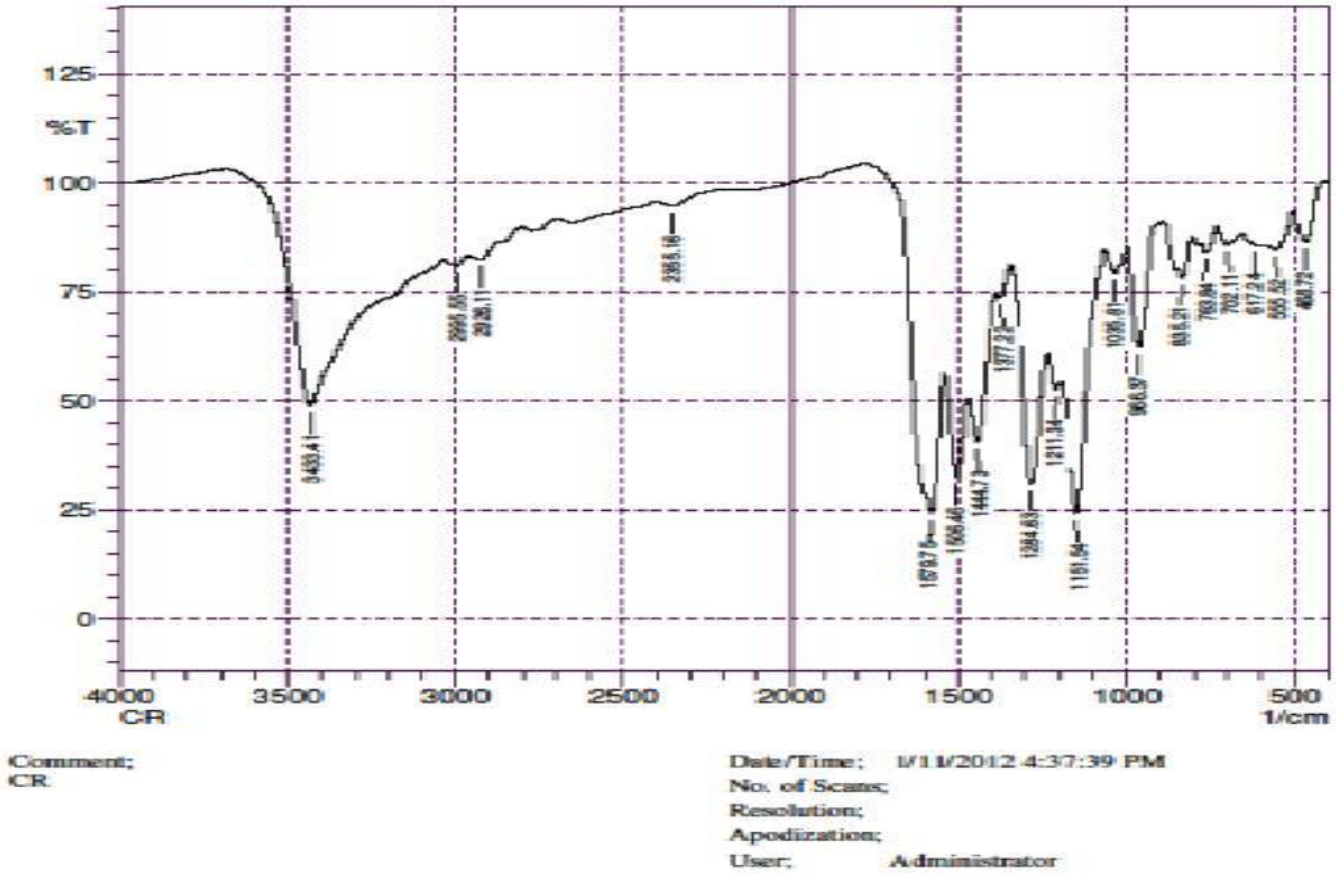
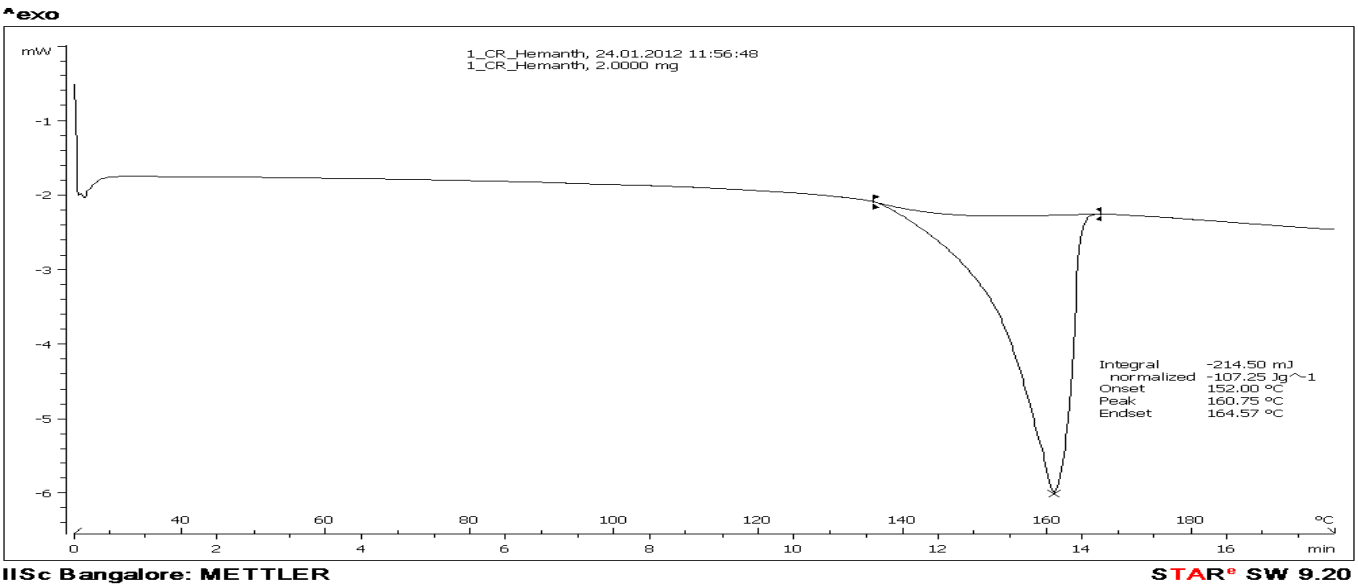
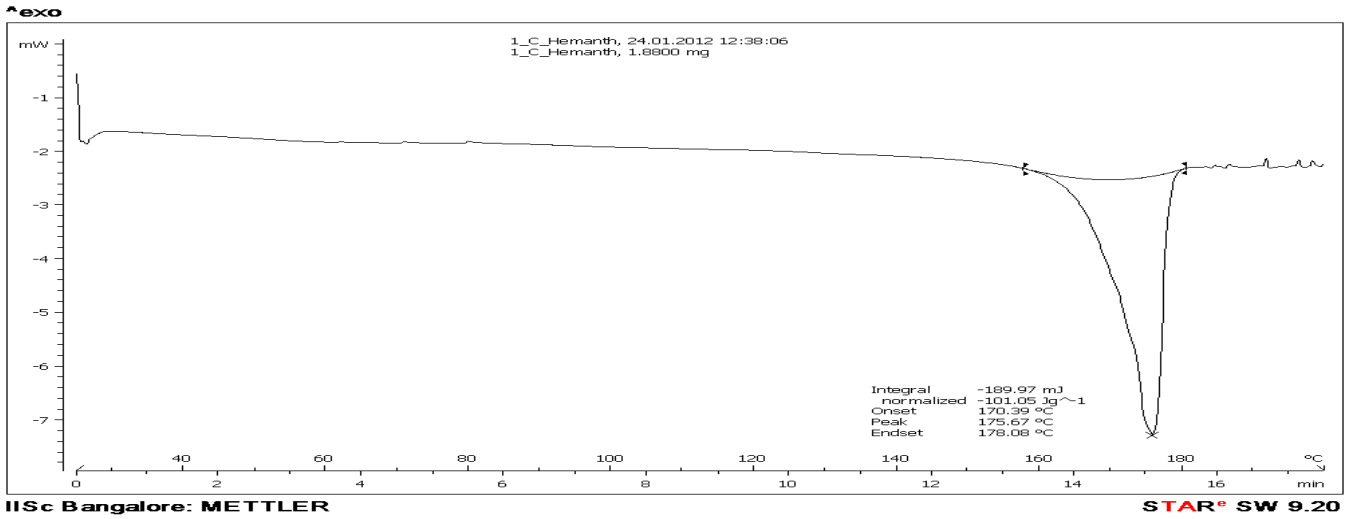


Figure 2. Comparative DSC thermo grams of curcumin (A), resorcinol (B) and prepared crystals (C)

(A)



(B)



(C)

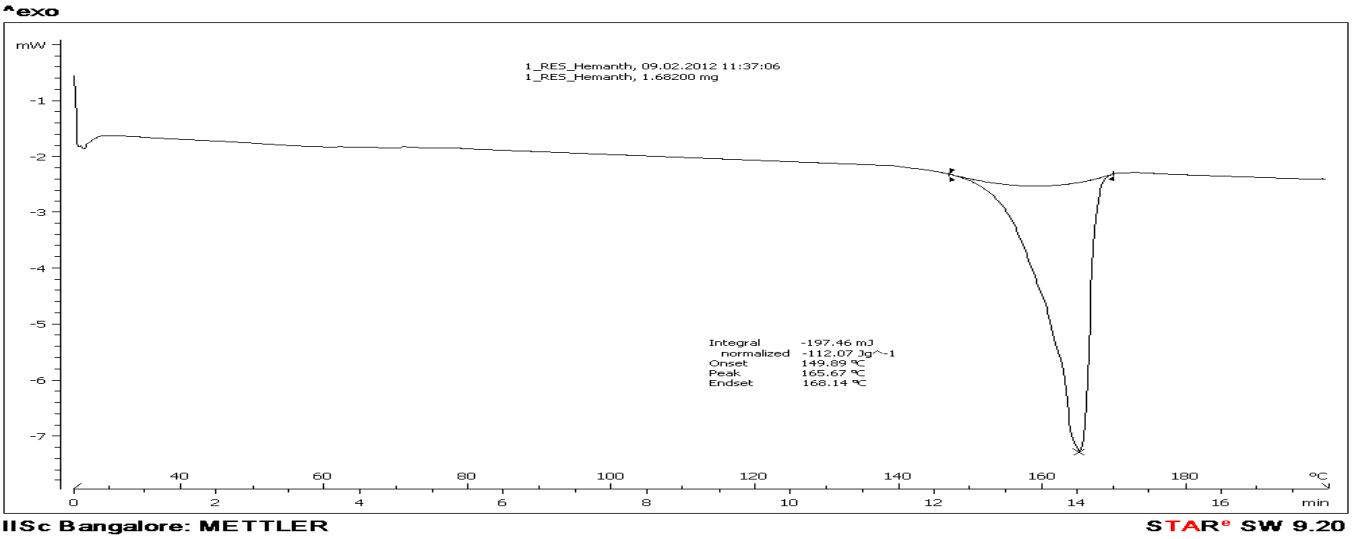
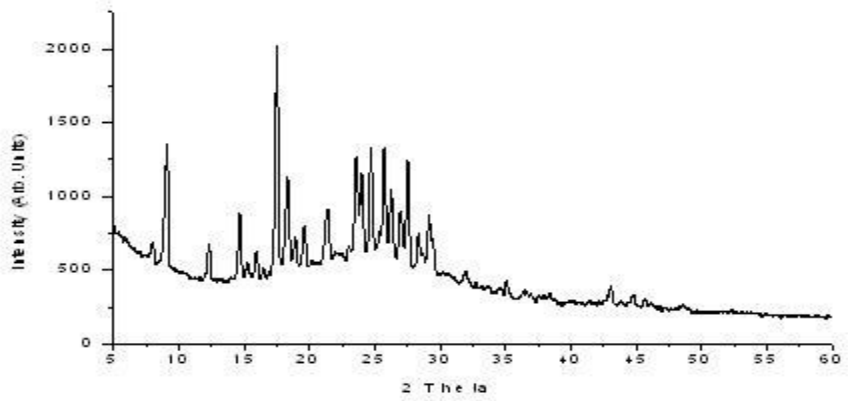
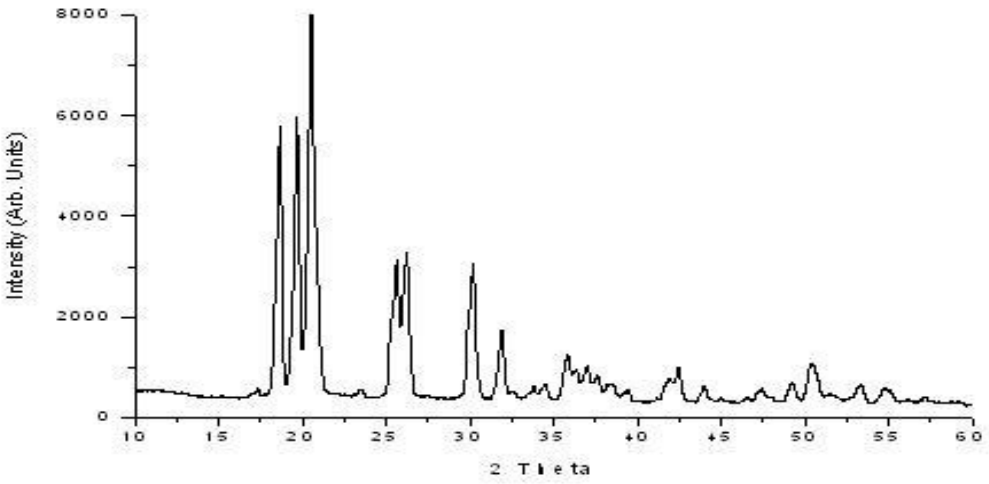


Figure 3. Comparative PXRD of Curcumin, Resorcinol and Prepared Crystals

Curcumin



Resorcinol



Prepared Crystals

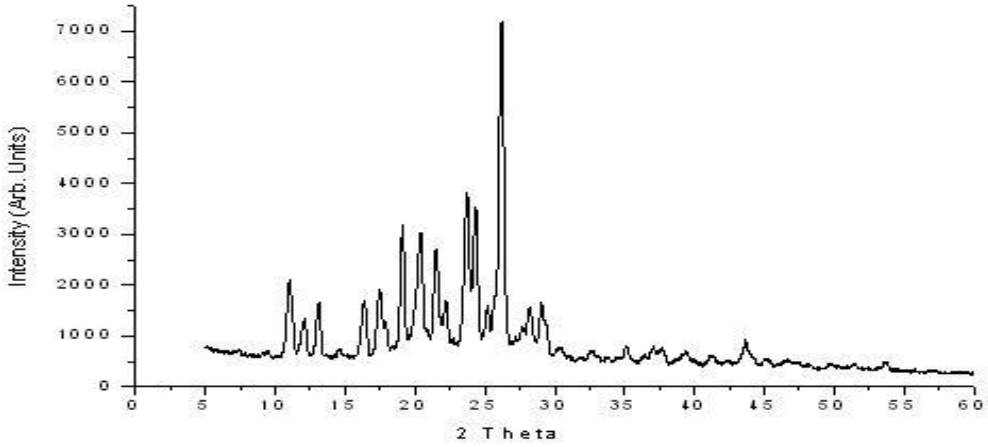
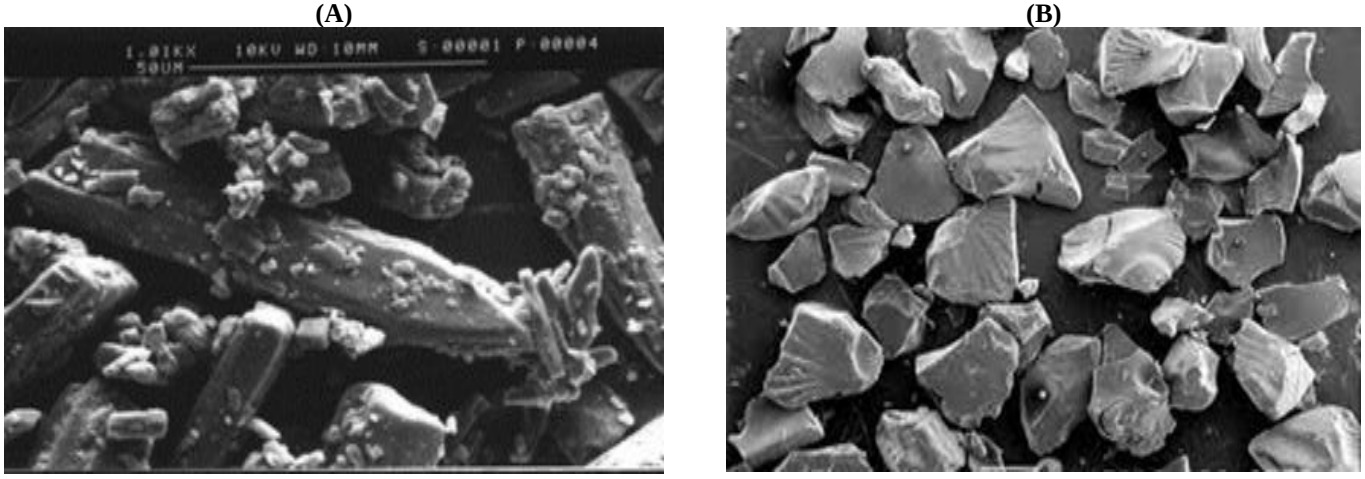


Figure 4. Comparative SEM of Curcumin (A), Resorcinol (B) and Prepared crystals (C)

(A) **(B)**



(C)

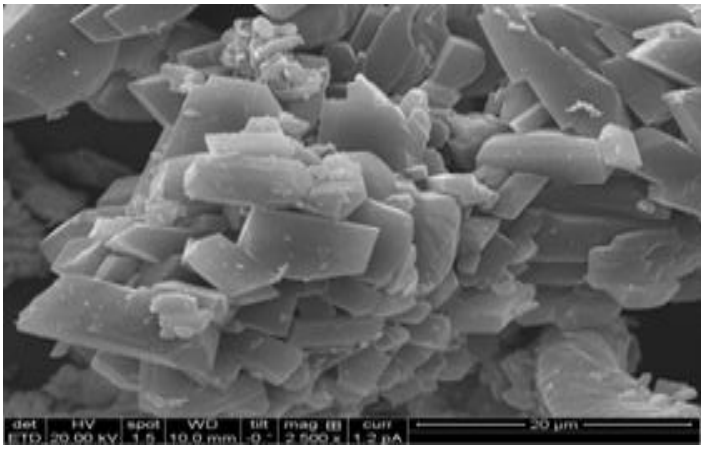
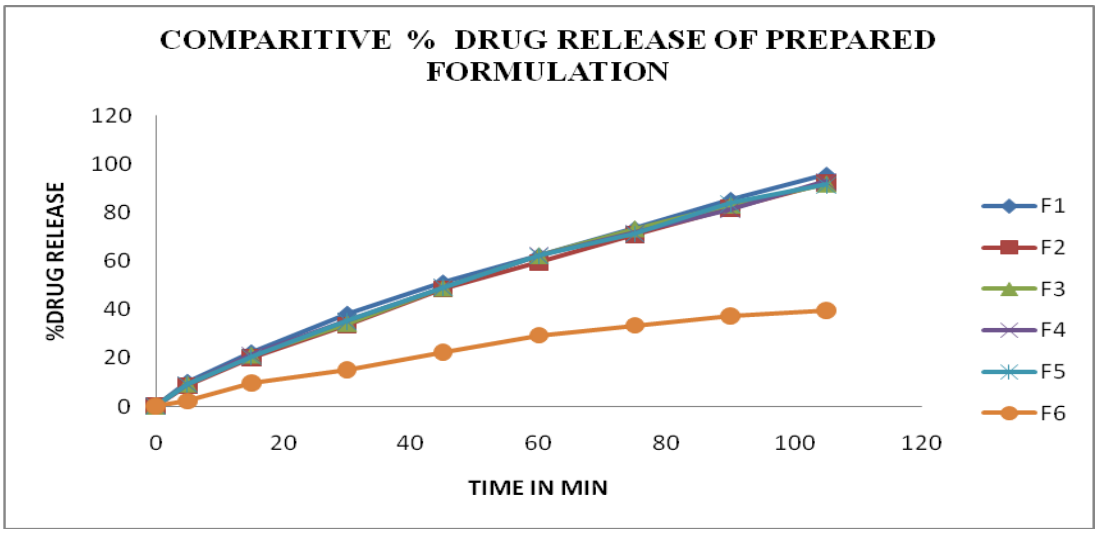


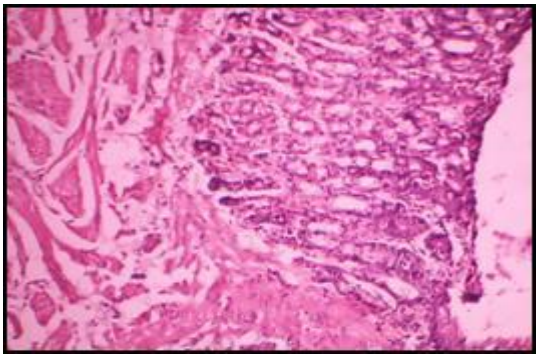
Figure 5. Comparative invitro dissolution



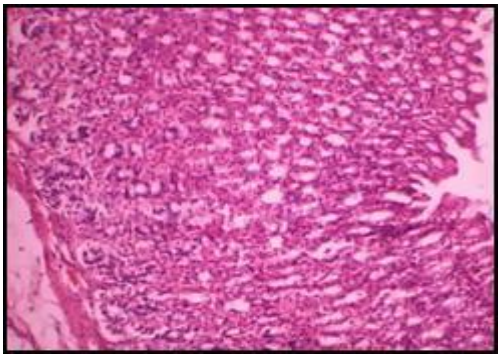
HISTOPATHOLOGY OF ETHANOL INDUCED GASTRIC ULCER

Figure 6. An effect of prepared crystals pre-treatment on ethanol -induced gastric ulcer in rats. Stomach tissue was stained with hematoxylin and eosin (100 x).

A



B



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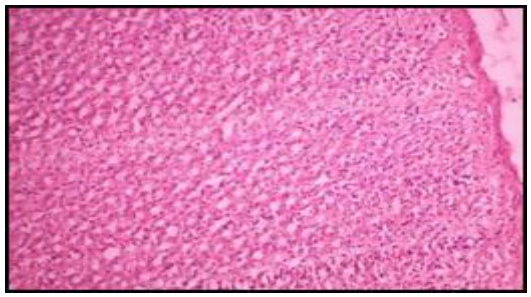
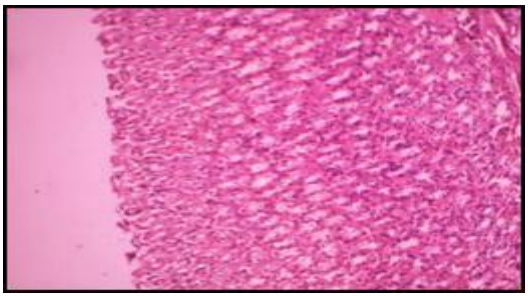


D



(A) Stomach after ethanol treatment,
(B) Stomach treated with CURCUMIN 100 mg/kg plus ethanol,
(C) Stomach treated Curcumin-Resorcinol crystals -100 mg/kg plus ethanol,
(D) Stomach treated with omeprazole-20 mg/kg plus ethanol.

Table 1. Formulation of tablets

S.NO.	INGRDIENTS	F1	F2	F3	F4	F5	F6
1	CO-CRYSTALS	54	54	54	54	54	200
2	LACTOSE	35	35	55	15	35	0
3	SODIUM LAURYL SULPHATE	35	35	15	55	35	0
4	PVP30	15	35	35	35	55	0
5	CARBOXY METHYL CELLULOSE	55	35	35	35	15	0
6	MAGNESIUMSTEREATE	4	4	4	4	4	0
7	TALC	2	2	2	2	2	0
TOTAL		200	200	200	200	200	200

Table 2. Comparative FTIR stretching vibrations (cm-1) of pure drug, resorcinol and prepared crystals

Sample/Peaks	Pure drug	Resorcinol	Crystals by L.A.G. Method
O-H	3504.77	3255.95	3433.41
C=O	1614.47	---	1506.46
Aromatic C=C	1585.54	1614.47	1444.73
Phenol C-O	1440.87	1296.21	1211.34
Enol C-O	1274.99/1197.83	---	1151.54

Table 3. Evaluation Chart of Tablet

Formul- ations	Weight variation in mg ± S.D	Thickness in mm ± S.D	Diameter in mm ± S.D	Hardness In Kg/cm^2 ± S.D	Friability (%)	Drug Content (%) ± S.D
F1	200.1± 0.05	3.0±0.01	1.01± 0.014	4.0 ± 0.16	0.25	99.36 ± 0.04
F2	200.8 ±0.01	3.1±0.03	1.01± 0.027	3.9 ± 0.13	0.29	96.41± 0.03
F3	200.5± 0.02	3.1±0.02	1.00± 0.015	3.8 ± 0.12	0.36	97.64± 0.01
F4	200.3±0.04	2.9±0.02	1.01± 0.015	3.9 ± 0.12	0.35	97.85± 0.03
F5	200.2±0.01	3.1±0.02	1.02± 0.018	3.9 ± 0.15	0.36	96.63± 0.02
F6	200.7±0.04	3.0±0.03	1.02± 0.016	3.8 ± 0.10	0.35	98.24± 0.02

Table 4. Disintegration time

Formulations	Disintegration time(min) ± S.D
F1	4.5 ± 1.23
F2	5.0± 1.13
F3	4.9 ± 0.92
F4	5.2± 0.95
F5	5.1± 1.19
F6	5.2± 1.32

Table 5. Uniformity of dispersion

Formulations	Uniformity of dispersions	
	Residue remaining on screen	Result
F1	Nil	Pass
F2	Nil	Pass
F3	Nil	Pass
F4	Nil	Pass
F5	Nil	Pass
F6	Nil	Pass

Table 6: Comparative *Invitro* Dissolution Study of Prepared Formulation (Tablets)

S. No	Time interval	F1	F2	F3	F4	F5	F6
1	0	0.00	0.00	0.00	0.00	0.00	0.00
2	5	9.98±0.01	8.55±0.02	9.19±0.03	9.01±0.02	8.99±0.01	2.12±0.01
3	15	22.12±0.02	20.12±0.02	21.11±0.03	21.18±0.02	20.22±0.02	9.54±0.02
4	30	38.12±0.02	33.44±0.01	34.17±0.01	35.15±0.01	35.12±0.03	14.85±0.02
5	45	50.98±0.01	48.44±0.01	48.88±0.02	49.11±0.03	49.12±0.02	22.25±0.02
6	60	62.12±0.01	59.32±0.05	62.22±0.02	62.24±0.02	62.12±0.01	29.15±0.01
7	75	73.14±0.01	70.71±0.01	73.30±0.02	71.41±0.01	71.12±0.02	33.31±0.03
8	90	85.15±0.02	81.81±0.02	83.11±0.01	81.21±0.02	83.75±0.02	37.23±0.02
9	105	97.24±0.02	92.24±0.01	91.84±0.01	92.82±0.03	91.51±0.01	39.45±0.01

Table 7. Effect of prepared crystals in ethanol (8 ml/kg) induced gastric ulcer in rats

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (1% w/v CMC, 10 ml/kg b.w) p.o	20.67 ± 0.71	---
II	CURCUMIN (100mg/kg b.w) p.o	18.63 ± 0.33*	09.86
III	Curcumin-Resorcinol crystals (100mg/kg b.w) p.o	4.83 ± 0.17**	76.63
IV	Omeprazole (20mg/kg b.w) p.o	3.83 ±0.31**	81.47

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. $^{*}P < 0.01$ and $^{**}P < 0.001$ as compared to control (n = 6 in each group). b.w= Body weight

CONCLUSION

Crystallization technique improves physicochemical properties and there by a quality pharmaceutical can be achieved.. The crystallization processes improve the flowability, compatibility and cohesivity of the poorly soluble drugs. The main aim of present study is to increase the solubility of the curcumin by supramolecular approach. These were characterized by FT-IR, DSC, PXRD, SEM. Slight variation in the wave lengths of the functional group in the molecular complexes of FT-IR spectrum. The melting points obtained from the thermographs of molecular complex were different from that of the pure drug and the excipient which confirms the formation of the new crystal phases. New peaks were observed in the crystalline pattern. From In-vitro dissolution studies it shows similar dissolution profile, with the percentage release of pure drug shows 39.45 % for 105 min, while the crystals of tablet show 97.24%

From the in-vivo studies the antiulcer activity of curcumin was evaluated gastric ulcer disease at dose of 100 mg/kg (dissolved in saline solution).Therefore, the comparison between such recommended dose of curcumin and one of the proton pump inhibitors (PPIs) Omeprazole 20mg/kg is worth-while. In ethanol induced ulcer model, co-crystal at a dose of 100 mg/kg body weight showed protective effect of 76.63%, whereas Omeprazole showed protection index of 81.47% at a dose of 20 mg/kg body weight. This study can be forwarded further for its improvement

REFERENCES

1. Aakeroy CB, Fasulo ME, and Desper J. Co-crystal or salt: Does it really matter? *Molecular Pharmaceutics*, 4, 2007, 317.
2. Schmidt BM, Ribnicky DM, Lipsky PE, Raskin I. Revisiting the ancient concept of botanical therapeutics. *Nature Chemical Biology*, 3, 2007, 360 – 366.

3. Callear SK. Preparation, characterization and structural analysis of salts and co-crystals of organic compounds. University of Southampton, School of Chemistry, PhD Thesis, 253, 2008.
4. Wenger M and Bernstein J. An alternate crystal form of gabapentin: A cocrystal with oxalic acid. *Cryst Growth Des*, 8, 2008, 1595-1598.
5. Basavoju S, Bostrom D and Velaga SP. Indomethacinsaccharin co-crystal: Design, synthesis and preliminary pharmaceutical characterization. *Pharm Res*, 25, 2008, 530-541.
6. Soanes C and Stevenson A. Oxford Dictionary of English, 2nd Ed., Oxford University Press: Oxford. 2003, 89-93.
7. Mizui T, Sato H, Hirose F, Doteuchi M. Effect of antiperoxidative drugs on gastric damage induced by ethanol in rats. *Life Sciences*, 41, 1987, 755–763.
8. Nguelefack TB, Watcho P, Wansi SL, Nguelta MM, Ngamga D, Tane P, Kamanyi A. The antiulcer effect of the methanol extract of the leaves of *Aspilia africana* (Asteraceae) in rats. *African Journal of Traditional Complementary and Alternative Medicines*, 2, 2005, 233-237.
9. Shah AH, Khan ZA. Gastroprotective effects of pretreatment with *Zizyphus sativa* fruits against toxic damage in rats. *Fitoterapia*, 3, 1997, 226–234.