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Nanoparticle Based Formulation of Quercetin and Evaluation of Its Anti-Ulcer Activity on Experimental Animal

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ABSTRACT- Object- The main objective of this study is to investigate the antiulcer activity of nanoparticle of quercetin on experimental animal.
Method- The quercetin nano-emulsion was prepared by high-speed homogenizer method.
Results- SEM which conforms the particles are in nano crystals is of crystalline in nature. The FTIR data show no chemical interaction between the drug and carrier. The present study shows that the pylorus ligation method showed significant decrease in the mean ulcer index in the pre-treated group as well as good degree of protection and so has the potential protects the stomach against ulcerogens. The combination of both drugs inhibited the aggressive factor and gastric acid secretions. The results was obtained from the present studies revealed that both drugs (quercetin and quercetin nano-emulsion) has reported for their anti ulcer property.
Keywords- FTIR, Nano-emulsion, Quercetin, Ulcerogens.

I. INTRODUCTION-

Nanotechnologies attracted significant attention in the recent researches. New technologies both in the preparation of the sample and in fabrication of device evoke on development of nano- science[1]. Nanocrystals are pure solid drug particals with size in the nanometer range. Nano-crystal is a material particle having at least one dimension smaller than 100 nanometers, and composed of atoms in either a single or poly-crystalline arrangement[2]. Nanoemulsions are a colloidal particulate system in the submicron size range acting as carriers of drug molecules. Their size varies from 10 to 1,000 nm. These carriers are solid spheres and their surface is amorphous and lipophilic with a negative charge. Magnetic nanoparticles can be used to enhance site specificity[3]. Peptic Ulcer is a common gastrointestinal disorder which is seen among many people; it is also cause by extensive use of nonsteroidal anti-inflammatory drug, strees condition and Helicobacter pylori invaded cells of gastric mucosa[4] Almost 5-15% of adult populations of the world are suffering from ulcer disease[5].

II. MATERAL AND METHOD-

Preformulation study of drugs

Determination of the physicochemical properties is the important steps are the preformulation studies before incorporating of the drug in its formulation. The properties of drug grealy affect the various parameters like method of preparation, compatibility study and pharmaco-kinetic parameters of the formulation. For the safety, effective and the stable formulation the pre-formulation studies is necessary. The selected drug quercetin was identified by various methods like organoleptic properties, Melting point Determination, Solubility, FT-IR Study.

Determination of pH

pH is an abbreviation for potential of hydrogen. The property of acidic or basic nature of substance is determined in terms of pH. It is determined by a scale called pH scale which ranges from 0-14. pH values below 7 are termed as being acidic while pH greater than 7 termed as basic in nature. pH value having 7 is termed as neutral. pH was established by the help of digital pH meter (361, Systronics). The electrode which was connected to the pH meter was cleaned with distilled H_2 O and made it dried up with the help of filter paper, then immerse the electrode and temperature probe in a beaker contain ointment formulations. After that wait for few minutes then note the readings of pH of samples which were displayed on pH meter. Experiment was performed in triplicates[6].

Determination of Melting points (M.P)

The M.P of drug quercetin and other other excipients was observed by the capillary MP apparatus. The melting point is the temperature at which a solid melts and becomes a liquid. In a typical procedure capillary tube was filled with tiny amount of drug from one end and another end blocked by heat and the drug loaded capillary

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inserted in one hole and thermometer inserted in another hole of the melting point apparatus and the melting temperature was determined.

Determination of Solubility

The solubility of the pure quercetin was determined with respect to these solvents system i.e. acetone, ethanol , dimethyl formamide (DMF), water, acetone, etc.

Preparation of quercetin nanoemulsion

To prepared the nanoemulsion, a primary emulsion was formulate by mixing 300 mg of quercetin and 3 g of Captex 355. This mixture was dispersed with 3 g of Tween 80 (by using stirrer for 1 hrs. at 65 C). After this, the mixture was homogenized by a high-speed homogenizer at 12,000 rpm for 4 min. After the homogenization, 6 gm of primary emulsion was added to earlier prepared mixture of 15 g of 5% sodium alginate and 1.5gm of soy lecithin, mixed on a Vortex mixer at 600 rpm for 3 mi. For nanoemulsion formulation, 0.5gm of primary emulsion mixture was dispersed into 100 mL of pure H_2 O and the pH was adjust to range from 4.5 to 9.0 with 0.1 N HCl and 0.1 N NaOH under mild stirring for 3 hrs. Then, the nano emulsions were stabilized for 24 hrs. at room temp. For the formation of animal diets. 30 g primary emulsion was formulated and dispersed into 2 kg of pure water and adjusted the pH to 8 and under a mild stirring for 3 hrs. for the formulation of quercetin loaded nanoemulsion was prepared as described above. After 24 h stabilization at room temperature, quercetin nanoemulsion was freeze dried before use[7].

FT-IR Study

FTIR spectroscopy is commanding tool for the identification of functional group present in the compound. It is a helpful tool to identify organic compounds, having polar functional groups (such as OH, NH, CH, etc.) with strong dipoles. It is very useful in the structural analysis of organic compounds, polymers, natural products etc. As every functional group present in a compound has an specific vibration, the IR spectra is seen as their fingerprints.

In this study, the FTIR analysis was performed to identify the potential interaction between the drug and the carrier. FTIR analysis was performed for Quercetin and quercetin nanoemulsion. For this function the samples were mixed with KBr and punched to a tablet applying hydraulic press. The FTIR spectra was recorded at 4000-400 cm-1 using FTIR Spectrometer (CDRI Lucknow)[8].

Scanning electron microscopy (SEM)

The SEM imaging of the sample is carried out by type of electron microscope which scans it with a high energy electron beam. In this when electron gets interacted with the atoms of the sample signals is produce which contains information of the sample morphology, its composition and other properties like electrical conductivity. It gives a better resolution than the optical microscope. In the present study, the Carbon coating of the materials was done by using JEOL- JEE-420 vacuum evacuator to make the sample conducting.Coating thickness was 20nm.SEM images were taken by using EPMA i.e. electron pro-micro analyser JEOL- J×A 8100.The average size range of the conjugate and its shape was determined[9].

Pharmacological study

Animals

Albino rats (150-200gm) of each sex and around the same age group were taken. They were kept in the departmental animal house at 26± 2 C and relative humidity 44-56% in polypropylene cages. The animals were exposed to alternate 12 hours of darkness and light each. Animal were provided with standard rodent pellet diet and the food was withdrawn 18-24 hr before the experiment through water was allowable ad libitum. All experiments were performed in the morning according to the current guidelines for investigation of experimental pain in conscious animals. The animal be authenticated by Institutional Animals Ethics Committee (IAEC), with REG No. is UIP/IAEC/NOV-2019.

Antiulcer activity by pylorus ligation induced ulcer

This is the animal model of gastric ulcers, originally developed by Shay in 1945[10]. Wistar rats weighing 150-200 g were fasted for 24 hours prior to pylorus ligation. The rate were anesthetized using ketamine by single syringe. The abdomen was open by a small midline epigastric incision of approximately 1 cm cut, and pylorus was exposed and stomach was untouched[11]. After this thread was tied around the pylorus part to stop the gastric secretion from stomach to duodenum, and ligated to avoid traction into the pylorus or damage to its blood supply, the stomach was carefully replaced and the abdominal wall was closed by interrupted

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sutures. The animals were deprived of both food and water, during postoperative period[12]. Ketamine injection and cervical dislocation and were sacrificed 6 hr after the operation. The stomach was excised considerately, keep the esophagus closed and opened from the greater curvature & collected the gastric juice. After this the stomach was washed with saline solution, and the stomach affixed on dissection chart. The ulcer index was considered and the lesion was counted. The collected gastric juice was centrifuged and various physicochemical, subjected to biochemical analysis to determine the free acidity, total acidity, volume, pH and histopathological examination[13].

Histopathological study

The animals were sacrifice by cervical disruption method. The animal was dissected and the stomach were expose and perfuse, with cold saline phosphate buffer of pH 7.4 for histopathological assessment as reported[14]. The stomach was taken out placed in containers separtly filled with formalin (10% v/v). Induction was done at 37 C under controlled condition for histopathological estimation. The histopathology was performed at United Diagnostics and Research, Prayagraj.

Statistical Analysis

Result expressed as the mean SEM for 5 animal. Analysis was perform by using One-way analysis of variation (ANOVA) and Turkey's Kramer multiple comparison test (software "Graph pad prism") was applied for determining the statical significance between different group. The result were observed, if $P<0.0001$.

III. RESULT AND DISCUSSION

1.Preformulation studies:

1.1 Determination of pH and Melting point:

Table 6.1

Sr. No.	Drug	pH	Melting Point (m.p)
1.	Quercetin	6.4	280 C

1.2 Determination of Solubility: Solubility in different solvents had been determined and affinities with various solvents are summarized in table 6.1.2. .

Sr.No.	Sample	Water	Ethanol	Methanol	Dimethyl Formaide	Acetone
1.	Quercetin	+	++	++	++	+++

2. FTIR Analysis

The FTIR analysis is one of the most main tools for the quick and efficient identification of chemical molecules. FTIR spectra was recorded to assess the compatibility of the pure drug and formulation compound and the FTIR spectra quercetin and quercetin nanoemulsion were ascertained in the FTIR test nanoemulsion. In this results showed that there is no chemical interaction The major characteristic peaks of querectin as (1100-1600 cm^{-1}) and OH phenolic bending (1200-1400 cm^{-1}) are present in free quercetin nanoemulsion. The presence of quercetin characteristic peaks on the quercetin nanoemulsion indicates the possibility of unchanged quercetin in quercetin nanoemulsion.

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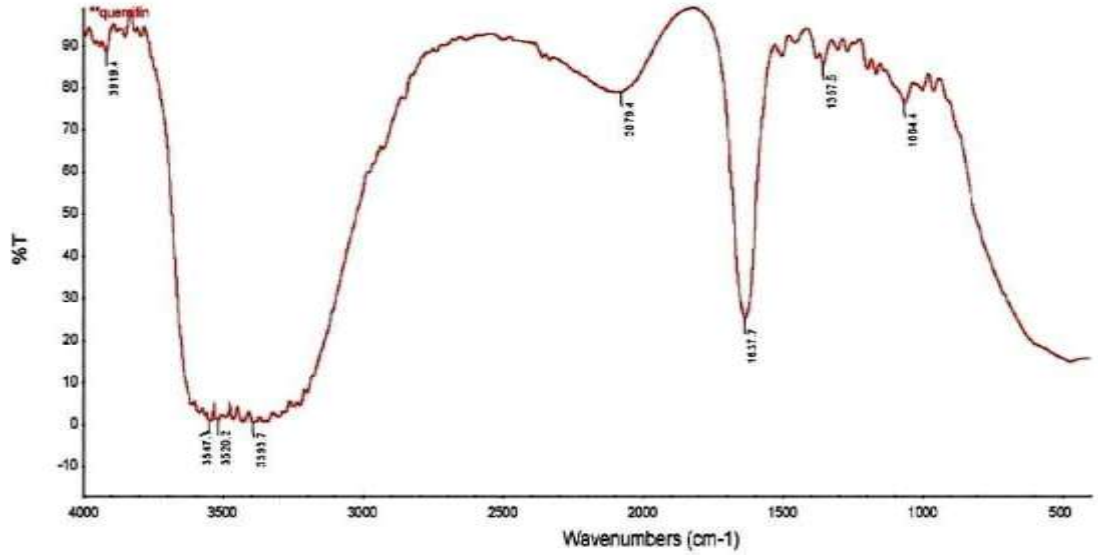

Figure:1 FTIR spectum of quercetin.

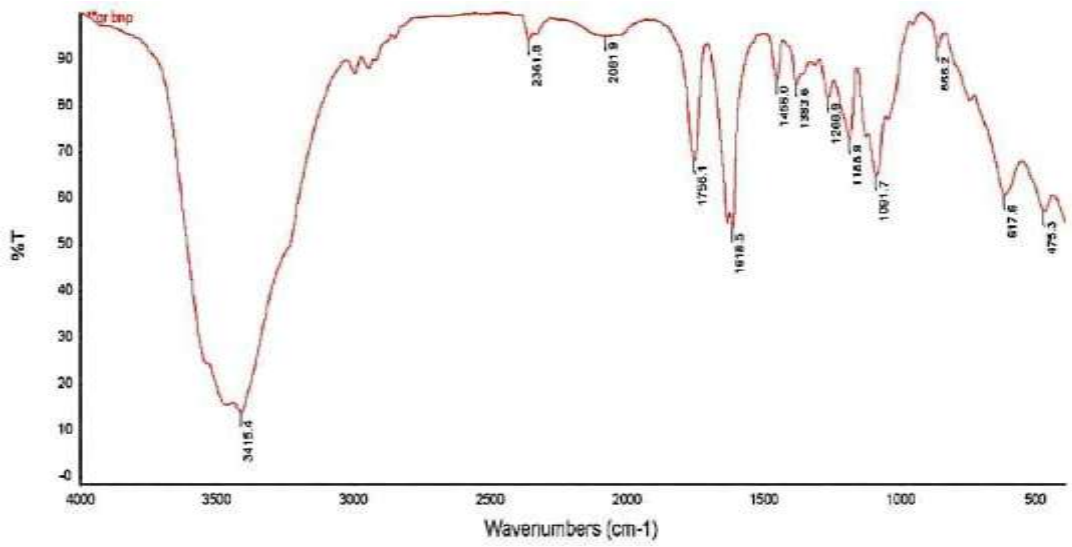

Figure:2 FTIR spectum of quercetin loaded nanoemulsion.

3. Scanning electron microscopy (SEM)

From SEM studies it had been found this the samples was crystal and around crystalline type. Crystal had been convinced by the dispersal of the solvent from the surfaced of the nanocrystals. The average size of the prepared nano-crystals was found to be in the range of 10-50nm. The shape of the nano-crystals is of crystalline in nature.

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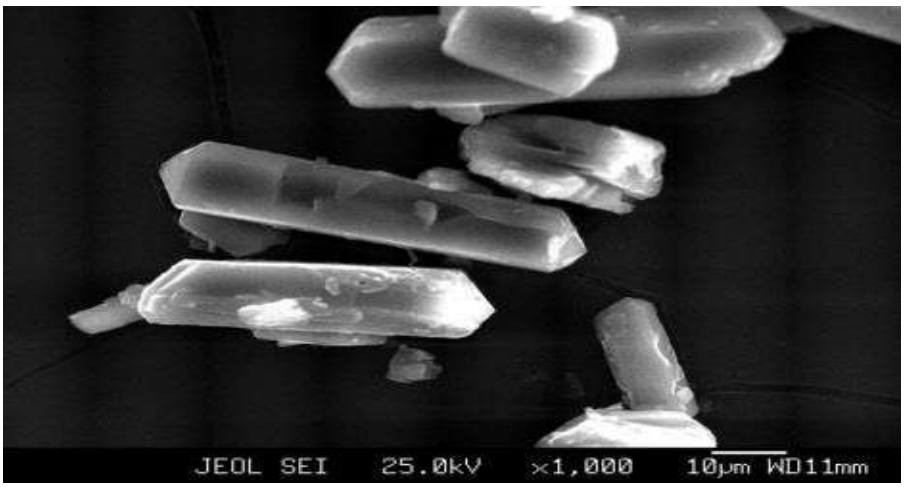

Figure:3 SEM image of NEC

4. Antiulcer Activity by pylorus ligation method

The effects of oral administration of formulation are given considerable response on ulcer index, gastric volume, free acidity total acidity and its pH. The quercetin suppress the ulcer index in a short limit but its high dose of Quercetin nanoemulsion are suppress ulcer index appreciably in ulcer index.

Table: 4. Antiulcer activity of pylorus ligation induced ulcer in rats.

Sr. No.	Parameters	Ulcer index	Gastric volume	Free acidity	Total acidity	pH
1.	Normal control	1.008±0.09	0.138±0.09	12.47±0.5	21.74±0.2	3.69±0.3
2.	Control	14.45±0.03	2.64±0.2	245.6±0.03	693.6±0.1	1.9±0.1
3.	Famotidine10mg/kg b.w	4.67±0.02***	0.903±0.038**	24.9±0.2**	42.65±0.1	4.45±0.1**
4.	Quercetine 5mg/kg b.w	8.63±0.01****	1.47±0.18*	61.98±0.1*	159.01±0.2	2.46±0.2*

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6.	**Quercetin Nanoemulsion 5mg/kg b.w**	6.2±0.05***	1.2±0.08***	35.434.1±0.3***	80.24.1±0.4***	4.1±0.2***

The values are the mean SEM of the six animals in each group. Statistical analysis was performed using one way ANOVA followed by tukeye's kramer multiple comparison test. **p<0.0001 compared with control.

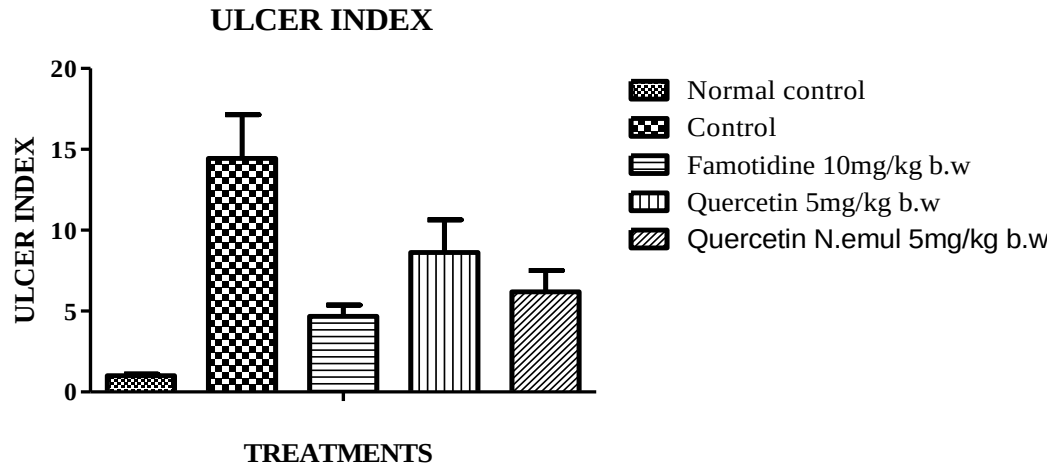

Fig.4 Effect of combination (quercetin and quercetin nanoemulsion) on ulcer index correlation between ulcer index and treatments.

The data was represented as Mean±SEM for six animal per group.P<0.0001considered significant comparted to control group. And the data when compared to standard for quercetin nanoemulsion less significant to quercetin.

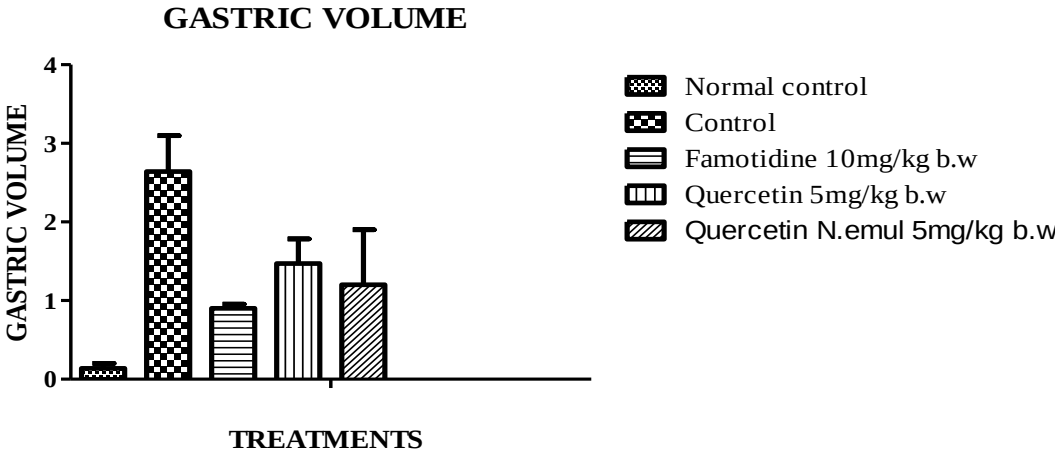

Fig. 5 Effect of combination (quercetin and quercetin nanoemulsion) on gastric volume correlation between gastric volume and treatment.

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The data was represented as Mean±SEM for six animals per group. P<0.0001considered significant comparted to control group. And the data when compared to standard for quercetin nanoemulsion less significant to quercetin.

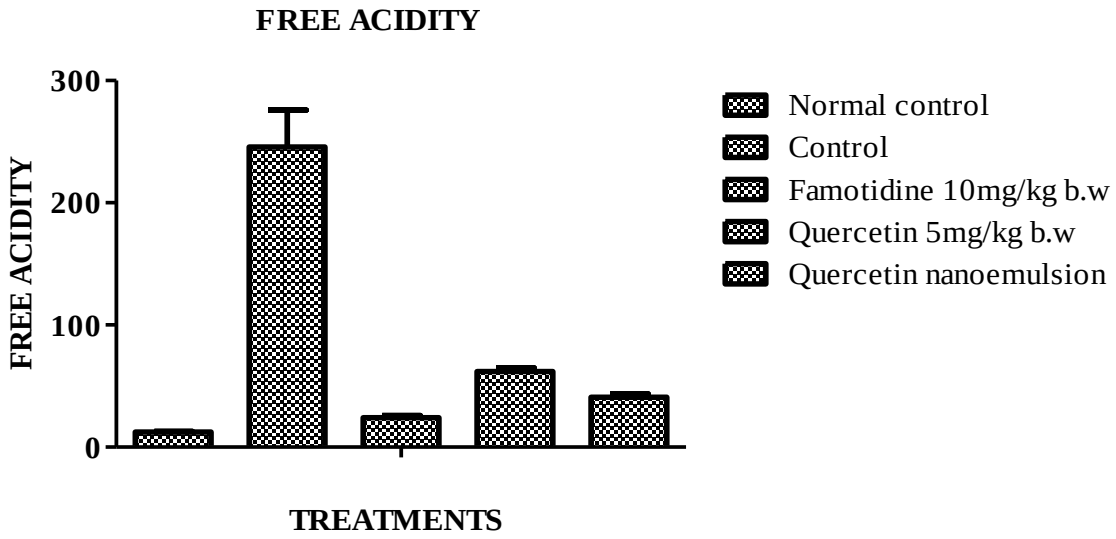

Fig. 6 Effect of combination (quercetin and quercetin nanoemulsion) on free acidity correlation between free acidity and treatment.

The data was represented as Mean±SEM for six animals per group. P<0.0001considered significant comparted to control group. And the data when compared to standard for quercetin nanoemulsion less significant to quercetin.

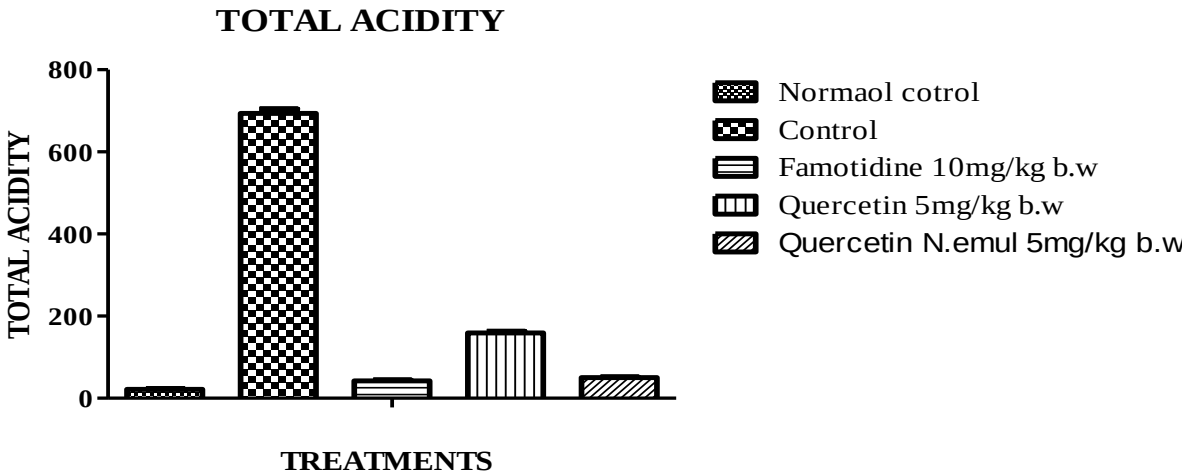

Fig. 7 Effect of combination (quercetin and quercetin nanoemulsion) on total acidity correlation between total acidity and treatment.

The data was represented as Mean±SEM for six animals per group. P<0.0001considered significant comparted to control group. And the data when compared to standard for quercetin nanoemulsion less significant to quercetin.

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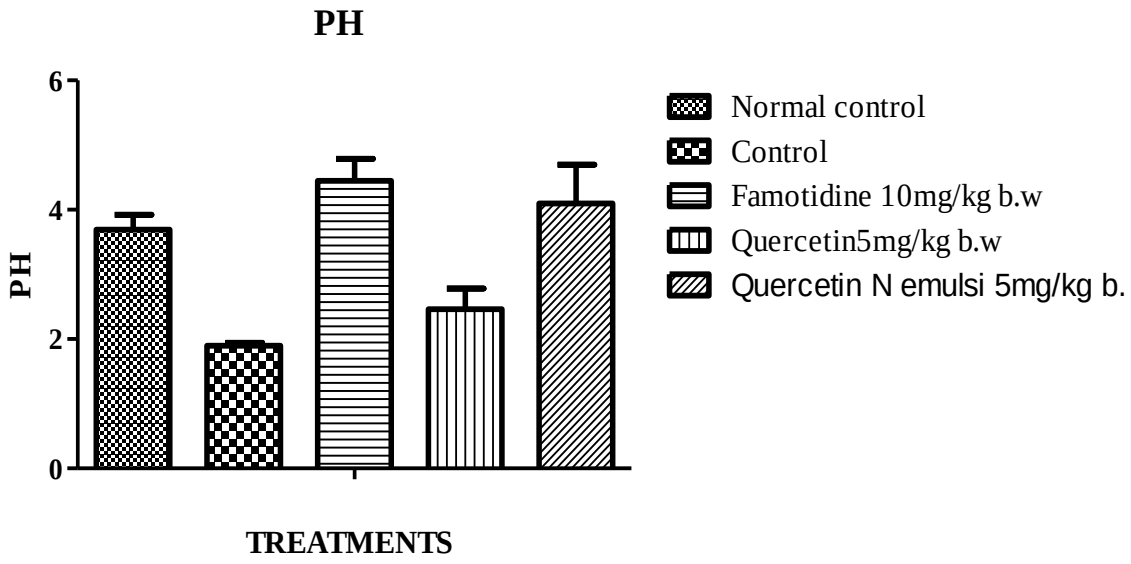


Fig.8 Effect of combination (quercetin and quercetin nanoemulsion) on pH with correlation between pH and treatments.

The data was represented as Mean±SEM for six animal per group. P<0.0001considered significant comparted to control group. And the data when compared to standard for quercetin nanoemulsion less significant to quercetin.

Histopathological examination study

Histopathology of stomach of normal control rats showed no ulceration, as no gastric lesion was found. It is confirmed by observation of transvers section of stomach where arrangement of cell is uniform throughout fig. 10. In control group of rats the cells are highly distorted due to ulcer. The arrangement of cells is highly ordered in the group with standard drug famotidine fig. 11. Quercetin treated rats showed less ulceration in campared to control groups but give more ulcer then combination and standard dose.fig.10 and 11. Histopathology of stomach of quercetin nanoemulsion rats showed much more less ulceration or no ulceration in comparision to quercetin fig. 12 and 13.

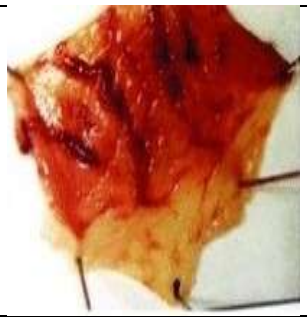
Histopathological examination study	
	
Fig.9 Normal control	**Fig. 10 Control**

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Fig. 11 Famotidine (10mg/kg) b.w

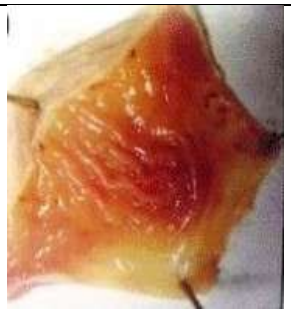

Fig. 12 Quercetin (5mg/kg)

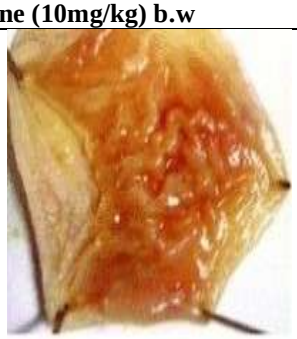

Fig. 13 Quercetin nanoemulsion (5mg/kg) b.w

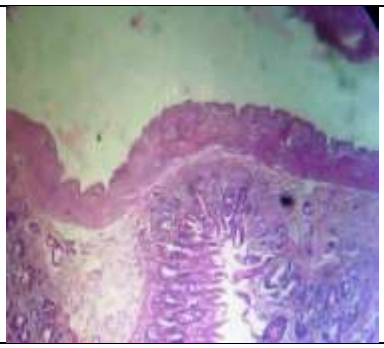

Fig. 12 Normal control

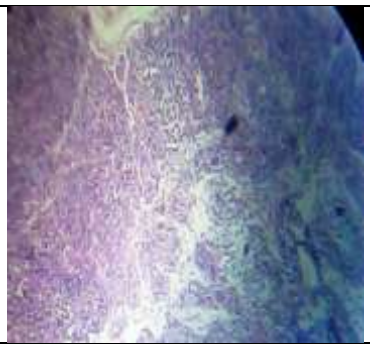

Fig. 13 Control

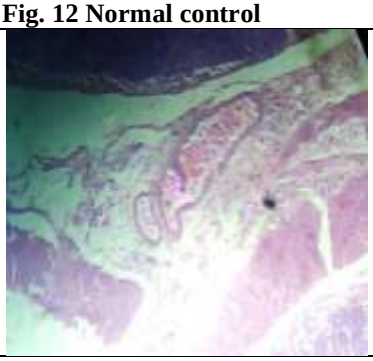

Fig. 14 Famotidine 10 mg/kg b.w

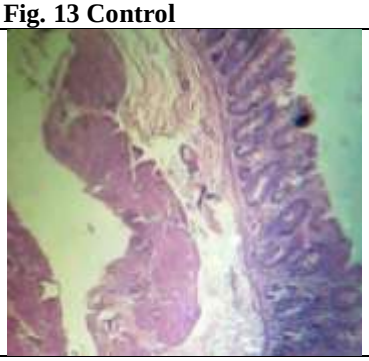

Fig. 15 Quercetin 5mg/kg b.w

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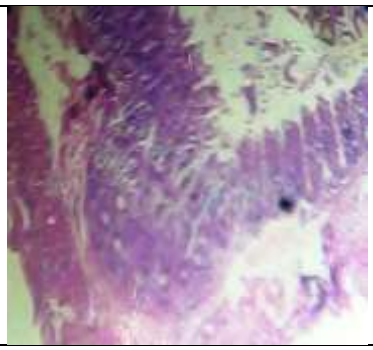

Fig.16 Quercetin nanoemulsion 5mg/kg b.w

IV. CONCLUSION

Nanotechnology is the science that deals with the matter at a scale of one billons of a meter and is also study of manipulating matter at the atomic and molecular scale. Nanotechnology is not providing a vehical through which we can deliver the higher reactive and biologically potent molecules but also over coming physical barriers to reach the targeted with conventional method. Nanoemulsion based formulations have been gaining important due to their targeted delivery, and increase bioavailability and controlled realise for the treatment of ulcer. In the present work the quercetin which is phenolic derivative. The quercetin nanoemulsion was prepared by high-speed homogenizer method, and it is characterized by SEM which conforms the particles are in nano crystals is of crystalline in nature.The FTIR data show no chemical interaction between the drug and carrier. Some studied have show that the drugs commonly used for peptic ulcer such as H_2 blockers famotidine, ranitidine etc. have change of drug interaction, adverse effect and increased incidence of relapse during ulcer therapy. This study shows that the pylorus ligation method showed significant decrease in the mean ulcer index in the pre-treated group as well as good degree of protection and so has the potential protect the stomach against ulcerogens. The combination of both drugs inhibited the aggressive factor and gastric acid secretions. The results was obtained from the present studies revealed that both drugs (quercetin and quercetin nanoemulsion) has reported for their anti ulcer property.

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