The Journal of Phytopharmacology 2015; 4(2): 90-96
Online at: www.phytopharmajournal.com


Research Article

ISSN 2230-480X
JPHYTO 2015; 4(2): 90-96
March- April


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# Fabrication, characterization and evaluation of hepatoprotective activity drug loaded flavono nanoparticle delivery system

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## Abstract

The liver is the second largest organ is tied to almost all the bodily processes as it is responsible for filtration and biotransformation of all incoming chemicals and fluids. Liver diseases are mainly caused by toxic chemicals, excessive intake of alcohol, infections and autoimmune disorders. Hepatotoxicity due to drug appears to be a common contributing factor. Medicinal plants are significant sources of hepatoprotective drugs and more widely used than allopathic drugs as hepatoprotective because these are usually inexpensive, better cultural acceptability, improved compatibility with the human body and minimal side effects. Flavonoids are natural products widely distributed in the plant kingdom and several Flavonoids such as quercetin, rutin, silymarin reported for their hepatoprotective activities. Nanoparticles are the submicron size particles diameter of around 200nm made up of biodegradable and non-biodegradable polymers. One of the important applications of nanoparticles in medicine includes effective drug delivery system. Hence the aim of the study is to prepare single loaded Flavono polymeric nanoparticles and compare its hepatoprotective efficacy with pure drug. Flavono polymeric nanoparticles were prepared by solid dispersion method using Eutragit 100 and Sodium lauryl sulfate as a carrier and resultant nanoparticles was used for further characterization. In-vivo hepatoprotective efficacy testing was performed by ethanol induced hepatotoxicity in albino rat model evaluate the efficacy of prepared Single loaded Quercetin, Rutin and Silibinin polymeric nanoparticles in comparison with pure compound.Nanoformulation significantly elevated liver biomarker (SGPT,SGOT,ALT,ASP).The study concluded nanoparticle-assisted formulation significantly enhanced the solubility in turn it improve bioavailability in survival even toxin-induced hepatic damaged cells.

**Keywords:** Flavono Nano Particle, flavanoids, Hepatoprotective activity, Ethanol.

## Introduction

The liver is the heaviest gland of the body and after the skin is the second largest organ of the body.[1] The liver weights about three and a half pounds (1.6 kg). It constitutes about 2.5% of an adult's body weight.[2] It is present in the upper part of the abdomen that aids in digestion and removes the waste products and worn-out cells from the blood. Liver is connected to two large blood vessels which include hepatic artery and portal vein.[2] Liver diseases are the major medical problems faced by the people all over the world.[3] About 20,000 deaths occur every year due to liver disorders.[4] In Africa and in Asia, the main causes of liver diseases are viruses and parasitic infections, whereas in Europe and in North America, a major cause is alcohol abuse.[3] Liver diseases are mainly caused by toxic chemicals, excessive intake of alcohol, infections and autoimmune disorders.[5] Hepatotoxicity due to drug appears to be a common contributing factor. Liver is expected not only to carryout physiologicalfunctions, but also to protect against the hazardous of harmful drugs and chemicals.[6]

In recent years, there has been renewed interest in the treatment against different diseases using herbal drugs as they are generally non-toxic and World Health Organization has also recommended the evaluation of the effectiveness of plants in a condition where we lack safe modern drugs.[7] Flavonoids are natural antioxidants derived from the plant constituents are widely present in the human diet in the form of numerous edible fruits and vegetables such as onions, apples, berries and red grapes.[8] The mechanism of the anti-oxidant action of flavonoids involve the of transition metal ions responsible for oxygen activation via redox reactions.[9] Some flavonoids, including quercetin and Silibinin can protect cells and tissues against the effects exerted by reactive oxygen species. Their antioxidant activity results from the scavenging of free radicals and other oxidising intermediates, from the chelating of iron or copper ions, and from inhibition of oxidases.[10] The one of the important clinical application of flavonoids like Silibinin, quercetin and rutin are widely used as a hepatoprotective, anti-inflammatory and antifibrotics agent.[11-13] In spite of this wide spectrum of pharmacological properties, the use of quercetin in pharmaceutical field is limited due to its low aqueous solubility and instability in physiological medium.[14]

These properties of quercetin result in poor bioavailability, poor permeability, instability and extensive first pass metabolism before reaching the systemic circulation.[15]

Nanotechnology is the manipulation of matter on atomic, molecular and supra molecular scale. National Nanotechnology Initiative defined nanotechnology as manipulation of matter with at least one dimension sized from 1 to 100 nanometers.[16] Nanoparticles are the submicron size particles diameter of around 200nm made up of biodegradable and non-biodegradable polymers. Size of nanoparticles is measured in nanometres. One of the important applications of nanoparticles in medicine includes effective drug delivery system. Hence the aim of the study is to prepare single loaded flavono polymeric nanoparticles and compare its hepatoprotective efficacy with pure drug. Flavono polymeric nanoparticles were prepared by solid dispersion method using Eutragit EPO as a polymer and Sodium lauryl sulfate as a carrier and resultant nanoparticle was used for further characterization.[17]

## Materials and Methods

The following chemicals were obtained from commercial sources and used as received without any further purification. Quercetin (97%) & Rutin (97%) was purchased from Sigma Laboratories (Mumbai, India). Ethanol (99.9%) was purchased from Brampton (Ontario, Canada). Sodium Lauryl Sulfate was purchased from S.D Fine Chemicals (Mumbai, India). β Cyclodextrin and Eutragit EPO was purchased from Himedia Laboratories (Mumbai, India).

### Fabrication procedure

Flavono polymeric nanoparticles were prepared by solid dispersion method as per the protocol of Suk Hyung *et al*[18] with slight modification. List and quantity of ingredients used in the formulations were listed in table 1. Accurately 50 mg of Quercetin, Rutin and Silibinin was weighed and dissolved in 50 ml organic solvent under bath sonication to ensure complete dissolution. Sodium lauryl sulfate was accurately weighed as per table 1 and dissolved in distilled water under bath sonication to ensure complete dissolution. Subsequently, the organic phase containing Quercetin, Rutin and Silibinin was added to the aqueous phase containing sodium lauryl sulfate under sonication (40 kHz; Lark, India) for 10 cycles (5 minutes per each cycle). Flavono polymeric nanoparticles were formed spontaneously and turned the solution turbid followed by removal of residual solvent by increasing the temperature under reduced pressure. Resultant nanoparticle was used for further characterization. Optimization was carried out to assess the impact of various concentrations of sodium lauryl sulfate and β-Cyclodextrin on the average particle of flavono polymeric nanoparticles. Particle size and related parameters were measured using Nanosizer (Malvern Instruments 2000, UK). Average particles size below 200 nm with uniformity below 1 and Span below 1.5, are considered acceptable.

**Table 1:** Optimization of flavono polymeric nanoparticles

| Code | Quercetin (mg) | Rutin (mg) | Silibinin (mg) | Ethanol (ml) | EPO | SLS (mg) | β- Cd (mg) | Distilled Water (ml) |
|---|---|---|---|---|---|---|---|---|
| Nano1 | 50 | 50 | 50 | 50 | 25 | 25 | 0 | 50 |
| Nano 2 | 50 | 50 | 50 | 50 | 25 | 0 | 25 | 50 |
| Nano 3 | 50 | 50 | 50 | 50 | 25 | 50 | 50 | 50 |
| Nano 4 | 50 | 50 | 50 | 50 | 25 | 0 | 50 | 50 |
| Nano 5 | 50 | 50 | 50 | 50 | 25 | 50 | 0 | 50 |
| Nano 6 | 50 | 50 | 50 | 50 | 25 | 50 | 25 | 50 |
| Nano 7 | 50 | 50 | 50 | 50 | 25 | 25 | 25 | 50 |
| Nano 8 | 50 | 50 | 50 | 50 | 25 | 0 | 0 | 50 |
| Nano 9 | 50 | 50 | 50 | 50 | 25 | 25 | 50 | 50 |

### *In-vivo* Hepatoprotective Testing

*In-vivo* hepatoprotective testing was performed to evaluate the efficacy of prepared Single loaded Quercetin, Rutin and Silibinin polymeric nanoparticles in comparison with pure Quercetin, Rutin and Silibinin.[19]

### Animals

Healthy adult male Sprague Dawley rats (120-200 gram body weight) were procured and randomly assigned to 10 groups, each containing 6 animals in polypropylene cages layered with husk and maintained in a controlled room at a temperature (22±3°C) and light (12 hours light/dark cycle). Animals were allowed free access to water and standard pellet diet. Animals were cared in accordance with the "Guide for the care and use of laboratory animals" and study was conducted in accordance with CPCSEA (approval No:1012/c/12/CPCSEA).

### Methodology

The 75% Ethanol solution was prepared at a dose of 4 gm/kg. Animals (except animals in the control group) received a single dose of Ethanol 4gm /kg once a day through p.o route 1hr after administration of pure drug and single, loaded polymeric nanoformulation for 30 days. Animals were allowed to develop hepatotoxicity (Table 2). At termination day, animals were anesthetized and blood collected. Hepatotoxicity was identified by biomarkers.[17]

**Table 2:** Experimental Design

| Group | Treatment |
|---|---|
| 1 | Animals were treated orally with 1ml of distilled water which is served as Control |
| 2 | 75% ethanol (4gm/kg) to induce hepatotoxicity and received no other treatment which is severed as Ethanol control. |
| 3 | Animals were treated orally with pure quercetin (50 mg/kg of body weight). After one hr 75% ethanol (4gm/kg) was administered. |
| 4 | Animals were treated orally with pure rutin (50 mg/kg of body weight). After one hr 75% ethanol (4gm/kg) was administered. |
| 5 | Animals were treated orally with pure silibinin (50 mg/kg of body weight). After one hr 75% ethanol (4gm/kg) was administered. |
| 6 | Animals were treated orally with prepared quercetin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight). After one hr 75% ethanol (4gm/kg) was administered. |
| 7 | Animals were treated orally with prepared rutin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight). After one hr 75% ethanol (4gm/kg) was administered. |
| 8 | Animals were treated orally with prepared silibinin loaded polymeric nanoformulation (equivalent to 50 mg/kg of body weight). After one hr 75% ethanol (4gm/kg) was administered. |

**Evaluation of hepatoprotective efficacy**

Efficacy of the pure and prepared nanoformulation was assessed using biochemical marker enzymes such as SGPT, SGOT, ALP(Alkaline Phosphatase), Total Protein and Total Bilirubin.

**Sample collection and biochemical assays**

The blood samples obtained were collected into plain sample tubes and centrifuged at 2000 rev/min for 5 minutes at 20 °C to separate serum. Serum was carefully collected and kept in eppendrof tubes for the determination of the biochemical parameters.

**Change in Body Weight**

Animal body weight was measured once weekly from the starting day to end of the study in ethanol induced models.

**Histopathology Study**

Rats from all the treatment groups and control groups were euthanized by ketamine on the day 28. After gross observation, liver was collected and fixed in 10% Neutral Buffer Formalin.

**Statistical analysis**

The data was analysed by using One way ANOVA followed by Turkey's multiple comparison test, *P<0.05 and **P<0.001 compared with Toxic treated group; **P<0.05 compared with vehicle treated group; # P<0.01. P value 0.001 is considered as statistically significant. Values expressed as Mean ±SEM

## Results and Discussion

**Preparation of Flavano Polymeric Nanosuspension**

Flavono polymeric nanoparticle was prepared using solid dispersion method. Totally nine formulations were prepared and summary of characterization of nanoparticles were listed in table 3.

**Table 3:** Characterization of nanoparticles

| Code | Particle size (nm) | | | | | Specific surface area ($m^2/g$) | Span (<1.5) | Uniformity (<1) |
|---|---|---|---|---|---|---|---|---|
| | d (0.1) | d (0.5) | d (0.9) | SWM | VWM | | | |
| Nano1 | 63 | 119 | 237 | 106 | 176 | 56.8 | 1.456 | 0.779 |
| Nano 2 | 65 | 124 | 635 | 112 | 211 | 53.8 | 4.590 | 1.020 |
| Nano 3 | 65 | 125 | 612 | 112 | 206 | 53.4 | 4.365 | 0.956 |
| Nano 4 | 70 | 149 | 4051 | 139 | 1056 | 43.3 | 26.65 | 6.430 |
| Nano 5 | 70 | 154 | 1070 | 141 | 629 | 42.6 | 6.503 | 3.450 |
| Nano 6 | 66 | 132 | 2953 | 123 | 759 | 48.7 | 21.80 | 5.060 |
| Nano 7 | 69 | 141 | 714 | 128 | 270 | 47.0 | 4.574 | 1.250 |
| Nano 8 | 74 | 186 | 10055 | 173 | 3519 | 34.6 | 53.71 | 18.4 |
| Nano 9 | 67 | 140 | 6632 | 133 | 2249 | 45.0 | 46.97 | 15.4 |

**Particle size**

The study has confirmed that d (0.1), d (0.5) and SWM of all the nine formulations were < 75 nm, < 200 nm and < 175 nm, respectively. In case of d (0.9), formulation Nano1, Nano2, Nano3 and Nano7 were in the nanometer range and other formulations were above the nano range. However, average particles size the formulation Nano1, Nano2, Nano3, Nano5, Nano6 and Nano7 were in the nano range and other formulations were above the nano range. Out of nine formulations, Nano1 has produced minimum average particle size of 176 nm (Figure 1) with uniformity of 0.779. However, formulation Nano8 has produced maximum average particle size of 3519 nm with uniformity of 18.4 (Figure 2) and formulation Nano9 has produced maximum average particle size of 2249 nm with uniformity of 15.4 (Figure 3). Morphology of pure compound and nano formulation is given in Figure 4 and 5.

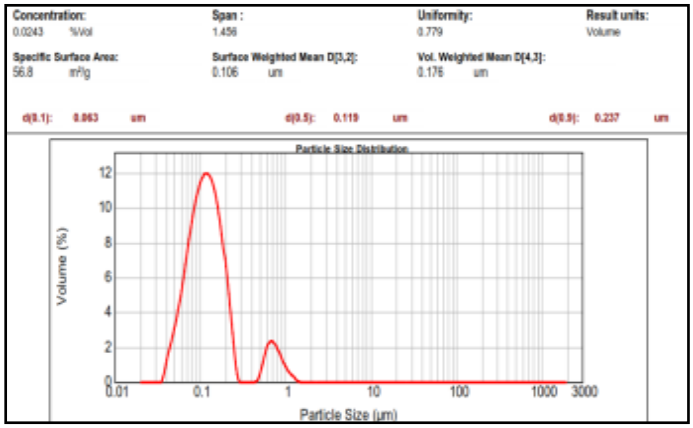


**Figure 1:** Particle size distribution of nano formulation 1

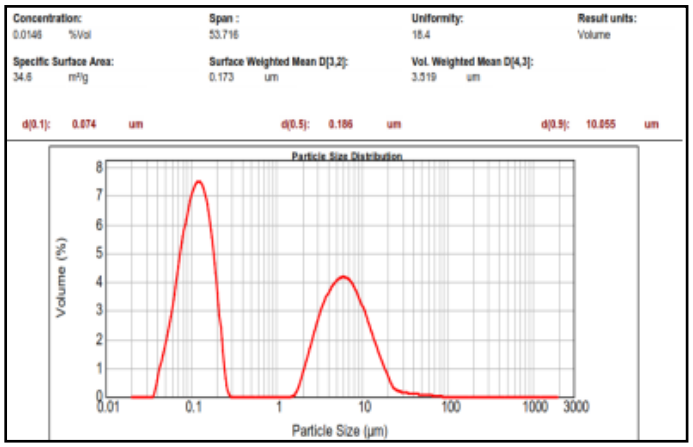


**Figure 2:** Particle size distribution of nano formulation 8

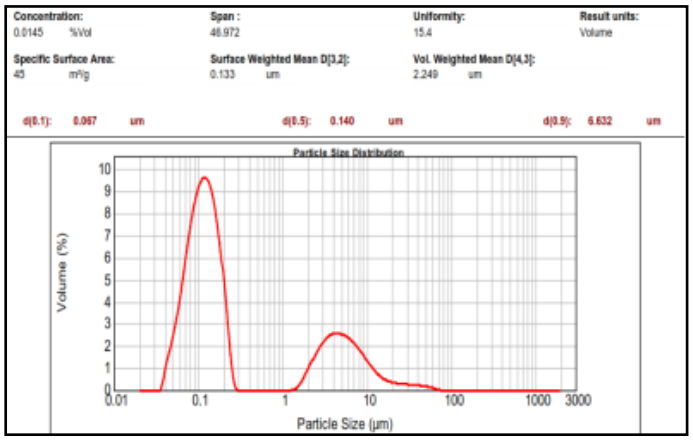


**Figure 3:** Particle size distribution of nano formulation 9

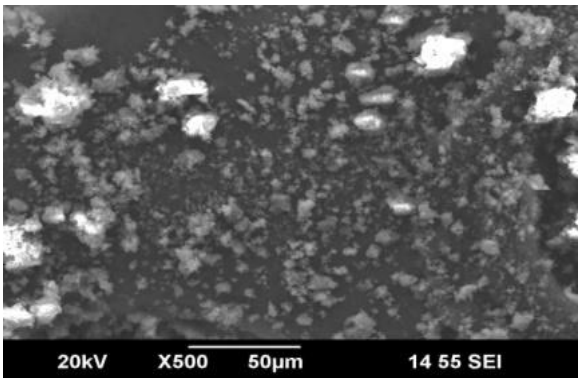


**Figure 4:** Morphology of pure compound

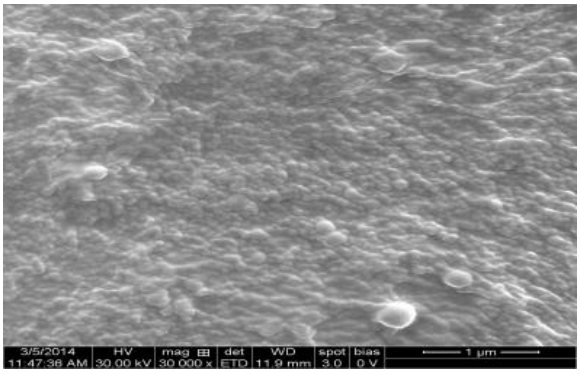

**Figure 5:** Morphology of nano formulation

**Span**

The span is the width of the distribution which is based on the 10%, 50% and 90% quantile and calculated based on the following formula Span = [d (0.9) - d (0.1) / d (0.5)]. Ideally, span should be less than 1.5. In the study, only one formulation (Nano1) had span less than 1.5. However, the maximum span was shown by formulation Nano8 with 53.71.

**Surface area**

Surface area of the particle is directly proportional to the aqueous solubility of the drug. Hence, significant increase in the particle surface area will significantly increases the drug aqueous solubility. The surface area of the pure Quercetin was 34.6 $m^2/g$ and in nanoparticle the surface area of the Quercetin and rutin dual loaded polymeric nanoparticle was increased significantly than the pure compound. The maximum increase in surface area of the Quercetin and rutin in nanoparticulate system was observed in Nano1 with 56. 8 $m^2/g$.

Optimization study of flavano polymeric nanoparticle has confirmed that formulation Nano1 had a minimum average particle size of 176 nm with a span of 1.456 and uniformity of 0.779 which satisfy all the acceptance criteria. Similarly, the surface area of flavano polymeric nanoparticle in Nano1 was higher than the pure Drug and other nanoparticle and hence, formulation was used for further studies.

**Description of Biochemical parameters in Ethanol induced model**

Ethanol induces number of deleterious metabolic changes in the liver. Intake of ethanol for a long time leads to the development of steatosis, alcoholic hepatitis and cirrhosis resulting in weight and volume changes. Results of the present study shows that the levels of SGOT, SGPT, ALP and Total Bilirubin were significantly increased in ethanol treated groups when compared with normal control group. While total protein is decreased in ethanol treated groups. Quercetin

nano, Rutin nano and Silibinin nano formulations show a marked decrease in SGOT, SGPT, ALP and Total Bilirubin when compared with toxic control group (Table 4).

**Table 4:** Biochemical Parameters

| Biochemical Parameters of Ethanol induced hepatoprotective model | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Dose mg/kg** | **SGOT(IU/L)** | **SGPT(IU/L)** | **ALP(IU/L)** | **Total Bilirubin (mg/dl)** | **Total Protein (gm/dl)** |
| **Normal Control** | Vehicle | 76.5±3.30 | 26.89±1.25 | 102.78±2.78 | 0.6±0.03 | 8.09± 0.19 |
| **Toxic Control** | 4ml /kg | 144.87±5.8# | 118.85±1.85# | 158.49±1.07# | 2.24±0.00# | 3.23±0.08# |
| **Quercetin** | 50mg | 106.20±2.11 | 45.01±2.53 | 118.80±3.25 | 1.23±0.14 | 6.00±0.18 |
| **Rutin** | 50mg | 105.11±1.04 | 44.41±0.69 | 115.22±1.15 | 1.57±0.04 | 5.50±0.20 |
| **Silibinin** | 50mg | 108.21±2.01 | 39.25±1.89 | 117.65±1.59 | 1.34±0.11 | 6.89±0.12 |
| **Quercetin Nano** | 50mg | 86.75±2.00* | 27.81±2.50* | 110.11±5.43* | 0.68± .14* | 8.04±0.11* |
| **Rutin Nano** | 50mg | 85.21±4.00* | 25.47±1.27* | 112.00±1.00* | 0.75±0.11* | 8.14±0.25* |
| **Silibinin Nano** | 50mg | 85.14±1.1* | 26.62±3.01* | 113.51±4.20* | 0.84±0.21* | 7.91±0.24* |

Values expressed as Mean ±SEM; Number of animals in each group N=6 *P<0.0001

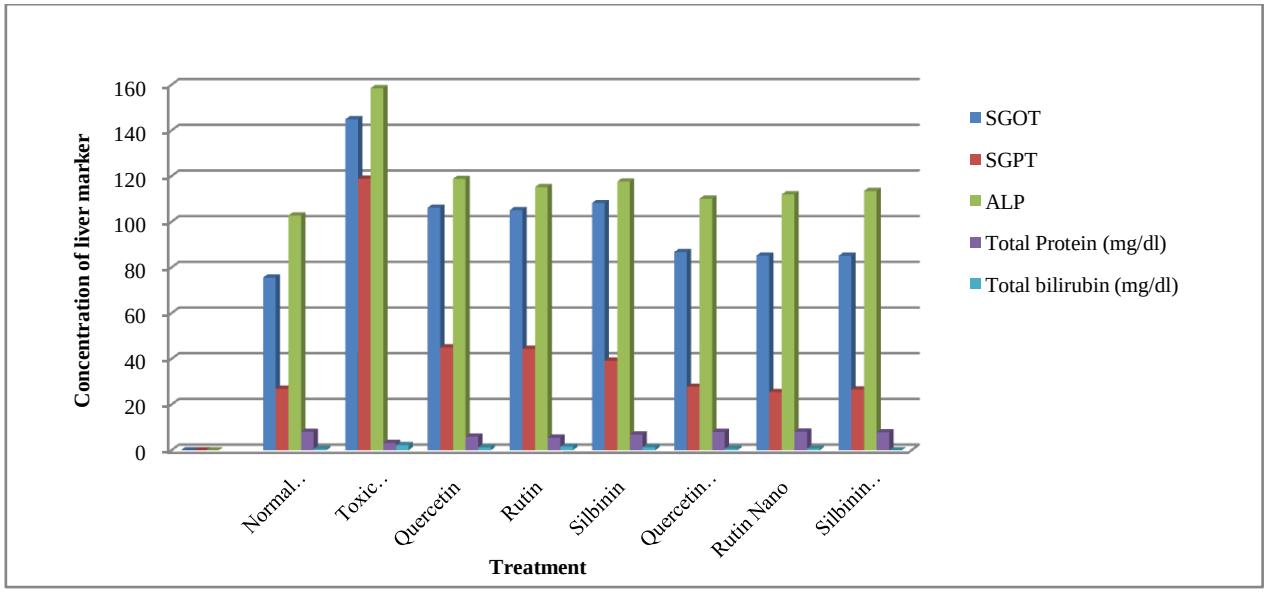


**Figure 6:** Graphical representation of Ethanol induced hepatoprotective model

**Body weight for Ethanol induced model**

Significant reduction of body weight was observed in the groups administered with Quercetin nano, Rutin nano and Silibinin nano groups, whereas the other groups show only slight reduction of body weight (Table 5).

**Histopathology of ethanol induced model**

Normal control shows the normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein.Toxic control exhibited severe hepatocyte degeneration, fatty changes and necrosis.Quercetin, Rutin and Silibinin shows mild hepatocyte degeneration.Quercetin nano, Rutin nano and Silibinin nano shows normal architecture with mild hepatocyte regeneration (Figure 7).

**Table 5:** Biochemical Parameters

| Treatment | Day | | | | | % Change In Body Weight |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | |
| **Normal control** | 160.25±3.24 | 169.01±2.4 | 183.95±4.1 | 200.32±5.6 | 224.00±1.2 | 39.78% |
| **Toxic control** | 158.31±5.60 | 170.92±5.7 | 188.00±3.6 | 210.11±3.9 | 247.1±2.8 | 56.08% |
| **Quercetin** | 162.01±2.10 | 172.55±3.5 | 187.12±5.0 | 206.00±2.1 | 230.15±3.2 | 42.05% |
| **Rutin** | 155.71±4.90 | 164.02±6.0 | 179.22±2.0 | 197.25±1.0 | 222.02±4.0 | 42.58% |
| **Silibinin** | 164.00±3.00 | 173.91±2.3 | 188.21±5.21 | 206.30±5.6 | 230.25±2.0 | 40.39% |
| **Quercetin nano** | 145.00±2.7* | 155.71±4.3* | 171.03±3.02* | 189.23±3.8* | 215.91±5.2* | 48.90% |
| **Rutin nano** | 149.00±3.8* | 160.75±3.6* | 175.23±3.25* | 194.20±3.2* | 219.23±4.9* | 47.08% |
| **Silibinin nano** | 154.10±2.80 | 164.21±4.0* | 181.03±2.08 | 199.31±2.9* | 225.25±2.7* | 46.17% |

Values expressed as Mean ±SEM; Number of animals in each group N=6 *P<0.0001

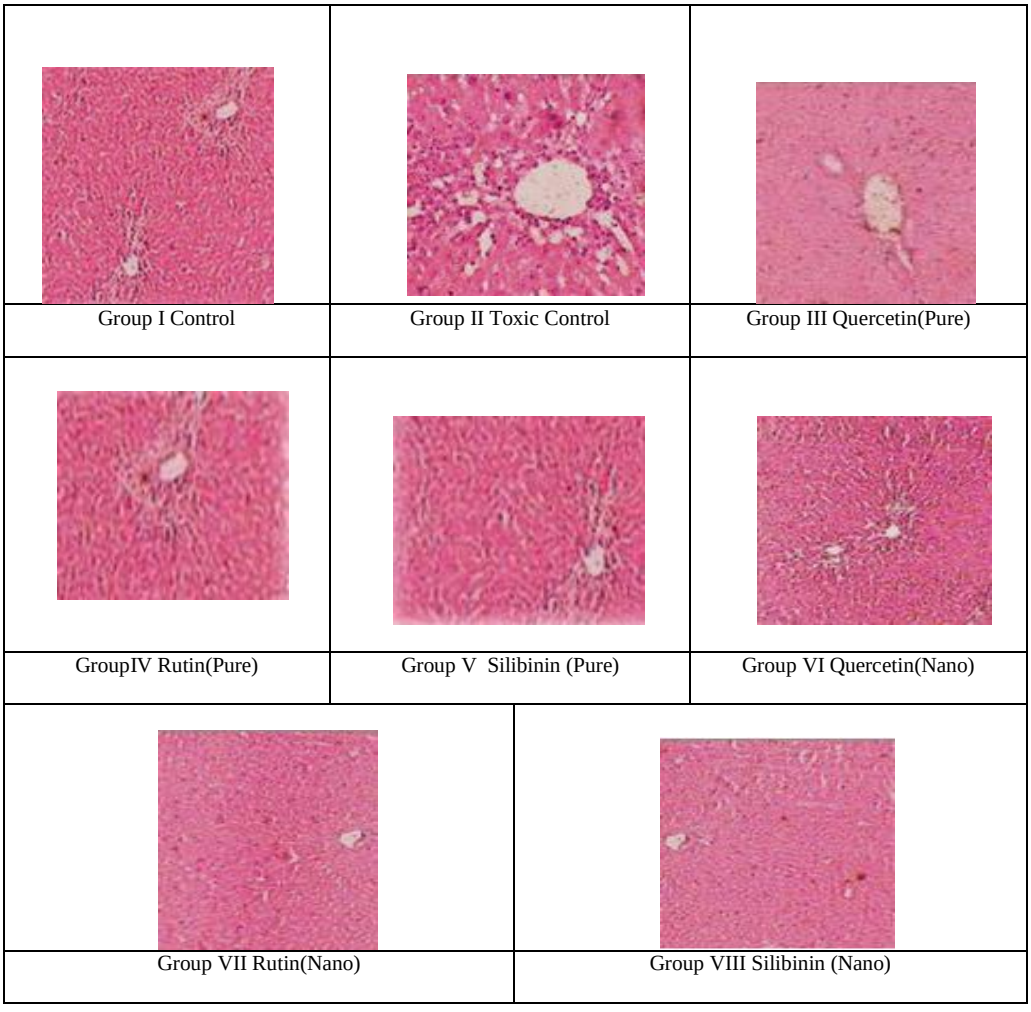


**Figure 7:** Histopathology of ethanol induced model

## Conclusion

A new nanoparticle delivery device for Quercetin, Rutin and Silibinin in EPO was successfully Fabricated, Characterized and protective properties against ethanol -induced hepatotoxicity were established. Increased Single loaded Nano formulation of quercetin, rutin and silibinin-induced rapid regeneration of hepatic Bio markers levels was demonstrated; along with down regulation of serum enzyme parameters, significantly increase in hepatocytes even when administered after toxin-induced hepatic damage. This appeared possible due to nano particle system improved solubilization increases intra and paracellular uptakes in turn it increases bioavailability of Single loaded Nano formulation of quercetin, rutin and silibinin.

**Acknowledgement**

The author thank to Dr.C.Moorthi and R.Sivasakthi for providing support for characterization of nanoparticle.

**Conflict of interest:** Authors declare no conflict of interest.

**Author Contribution:** Dr.S.Mohan research supervisor and Nanthakumar.N research coordinator.

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