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BIOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF QUERCETIN POWDER AND ITS NANOPARTICLES

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ABSTRACT

Liver diseases including cirrhosis, hepatitis, and hepatocellular carcinoma have alarming worldwide incidences of morbidity and mortality. Another compound from the natural origin that has been examined in the context of the liver protection are flavonoids like quercetin, characterized by potent anti-inflammatory and anti-oxidative action. In the present investigation, quercetin has been encapsulated in quercetin nanoparticles (QNPs) to assess hepatoprotective activity in chemically induced liver damaged rats and compare it with quercetin powder (QP) to achieve the desired therapeutic efficacy of quercetin. Quercetin nanoparticles were prepared using solvent evaporation technique while the structural confirmation of the nanoparticles was achieved using FTIR spectroscopy. Four groups of male Wistar rats were created: first group was for control group, second group was treated with QP, third group was treated with QNP and the last group was treated with liver toxic carbon tetrachloride (CCl_4). For the assessment of liver tissue injury and reparative processes specific histopathological examination was made together with evaluation of chemical biomarkers such as ALT, AST, and ALP. The antioxidant effect of both quercetin formulations was further evaluated and the haemostatic profile of the QNPs was compared to that of QP. The results indicated that, in contrast with quercetin powder, quercetin nanoparticles enhanced the hepatic protection notably. Hence, it is clear that QNPs showed a better decrease in ALT and AST levels, higher antioxidant properties and greater normalization of liver enzymes. Histopathological changes indicated that QNPs enhanced the regeneration of tissue along with the reduction of necrosis and inflammatory response as well as provided better amelioration of CCl_4-induced hepatic toxicity. Besides, QNPs showed higher haemostatic property compared to QP indicating shortened clotting

time. In conclusion, quercetin nanoparticles can serve as an effective solution to improve the hepatoprotective and bioavailability profile of quercetin that possess potential application in liver diseases treatment. The outcomes suggest about the actualization of pharmacokinetic limitations of natural chemicals like quercetin and propose the positive utilization of nanoparticle-based drug delivery systems.

Keywords: *Hepatoprotection, Antioxidant Activity, Quercetin Nanoparticles, Liver Diseases, And Nanotechnology*

INTRODUCTION

The liver plays a central role in maintaining physiological balance through its functions in detoxification, metabolism, and biosynthesis. It is still very vulnerable to harm from drugs, alcohol, infections, and pollutants in the environment, though (Fatima et al., 2025). A major global health concern, liver diseases like cirrhosis, hepatitis, and hepatocellular carcinoma frequently result in systemic complications. These conditions highlight how urgently effective hepatoprotective medications are needed. A naturally occurring flavonoid that is widely present in fruits and vegetables, quercetin has demonstrated significant promise because of its hepatoprotective, antioxidant, and anti-inflammatory qualities (Azeem et al., 2023; Yousaf et al., 2025). When taken in its traditional powdered form, its poor aqueous solubility, low gastrointestinal absorption, fast metabolism, and general low bioavailability have restricted its clinical use despite its therapeutic potential. Recent developments in nanotechnology have shown promise in resolving these pharmacokinetic issues (Qasim et al., 2024). Quercetin's solubility, stability, and bioavailability can all be greatly improved by encapsulating it in nanoparticles (QNPs) (Basheer et al., 2025). By reducing the compound's particle size to the nanoscale, nanoparticles increase surface area and facilitate quicker absorption and dissolution. Additionally, they improve targeted delivery to critical organs like the liver, prevent premature degradation of the active molecule, and permit controlled and sustained drug release. In light of this, the current study uses both in vitro and in vivo models of chemically induced liver damage to assess and compare the hepatoprotective effectiveness of quercetin powder (QP) and quercetin nanoparticles (QNPs) (Muhammad et al., 2020). Along with liver enzyme activities and histopathological changes in hepatic tissues, the study focusses on evaluating important biochemical indicators of liver function and oxidative stress, such as MDA, GSH, and LPO levels (Khan et al., 2024). Since there are currently few direct comparisons between QP and QNPs in liver protection, this study fills a significant knowledge gap. The goal of the study is to shed light on the different pharmacological reactions of these two formulations in order to improve the therapeutic delivery of quercetin. In addition to improving quercetin's use in hepatology, the results should support the potential of delivery systems based on nanoparticles to advance the clinical use of natural substances with low bioavailability. In the end, this research could help create more focused and efficient treatments for liver diseases, opening the door for advancements in pharmacotherapy based on natural products (Abid et al., 2024).

METHODOLOGY

MATERIALS

The other materials used in the study include: Quercetin, ethyl alcohol, acetone, polyvinyl alcohol (PVA), Deionized water and Triton X- 100. Peach flavored cotton dressing and placebo substances were used for the control groups while albino rats were used for the experiments.

PREPARATION OF QUERCETIN NANOPARTICLES BY SOLVENT EVAPORATION

The formation of nanoparticles using quercetin by the solvent evaporation technique was carried out by the following sequential process: To start, quercetin is dissolved in an organic solvent like ethanol and hence, create an organic phase. At the same time and under stirring an aqueous phase is obtained by mixing polyvinyl alcohol (PVA) with distilled water at a high temperature to dissolve the polymer completely. The aqueous PVA solution is prepared separately and then the organic phase containing quercetin is added dropwise to the aqueous PVA solution under magnetic stirring and at room temperature, this results in the formation of an emulsion. This emulsion is then sonicated at 40 kHz for 30 minutes in order to better disperse quercetin and therefore reduce the particle size of the compound. Subsequently, the solvent evaporation process is done by stirring the emulsion at room temperature or under reduced pressure to eliminate the organic solvent and quercetin nano-particles would be dispersed in aqueous phase. The nanoparticles are then centrifuged at 10,000 rpm for 20 minutes and washed several times with distilled water so as to remove any PVA and solvent. Last of all, the purified nanoparticles are freeze dried so that a dry powder is obtained out of it which can be used in various forms in several applications.

FTIR:

Characterisation of the functional groups in the extracts was done using FTIR spectroscopy. The recorded spectra were in the region of 4000-400 cm-1 with a spectral resolution of 4 cm -1. The final FTIR spectrum showed a spectrum obtained after subtracting the background spectrum from the sample spectrum. The maxima in the spectra were determined and related to the various functional groups present.

LABORATORY ANIMALS

Male Wistar Albinos rats (200-250 g) were purchased from Multan, Pakistan. The animals were kept under light/dark cycle of 12/12 hours, humidity level of 60% and temperature of 25°C. They also received food ration on regular basis. To most of them water was available.

EXPERIMENTAL DESIGN

Four rats were put into each group and groups were randomly formed with total of four groups. The positive control in Group A was the rats that has not been administered on any drug. To produce hepatotoxicity, groups B, C and D were given CCl_4 (1mL/kg I. P.) for 14 days. Group B was used as reference group. Groups C and D were treated with quercetin powder and nanoparticles, respectively; serum and liver tissue samples of the rats were subjected to biochemical investigations to determine the hepatoprotective potentials of nanoparticles.

BLOOD AND SERUM SEPARATION

Five millilitres of blood was filled from the rat tails and the serum was definitely obtained after the spinning was done at 1500 RPM for a period of 10 minutes. The serum was kept at -60°C with a plan to perform another biochemical analysis on the serum later.

TISSUE HOMOGENATE

Liver tissues were gently homogenised in sodium-phosphate buffer saline which had a concentration of 10 mM to have a concentration of 25% homogenate. Using a centrifuge at 1500 rpm for 15 min the homogenate was separated and the supernatant was collected and preserved at -60°C till the biochemical estimations were done.

DETERMINATION OF LIVER ENZYMES LEVELS

An ELISA kit from Biomerieux, USA was used to assess the altitude of the animal's blood samples to follow the standard procedure to measure the blood level of ALT, AST & ALP.

HISTOPATHOLOGICAL EXAMINATIONS

The rats' livers were preserved in 10% formalin for one day. The rats' livers were preserved in 10% formalin for one day. After fixation, the tissues were treated with eighty grams of eighty percent ethyl alcohol and then washed with tap water. After that the specimens were washed in xylene for a while and then they were put in paraffin for one day at 50°C in hot air oven. After the fixation of the treated tissues in paraffin block, sections of about 4 µm thickness were made with the help of sledge microtome and thereafter placed on to the glass slides. These slides which were taken from paraffin blocks were deparaffinized then stained with haematoxylin and eosin and examined in a histology laboratory on two weeks, four weeks, and six weeks intervals.

STATISTICAL ANALYSIS

All the statistical analysis was done using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA). This was done using analysis of variance (ANOVA) and data were presented as mean ± standard deviation. Accordingly, the findings suggest that a p-value of less than 0. 05 was employed to determine a statistically significant result.

RESULTS AND DISCUSSION

FTIR

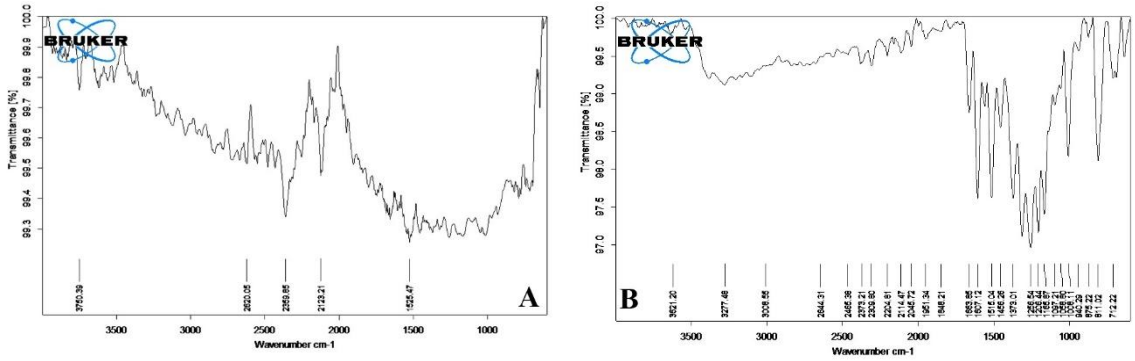

FIGURE 1:(A) FTIR OF QUERCETIN NANOPARTICLES SHOWS DISTINCT PEAKS (B) QUERCETIN POWDER'S FTIR SHOWS DISTINCT PEAKS WHICH CONFIRM THE TYPICAL FUNCTIONAL GROUPS PRESENT IN QUERCETIN

Different motive peaks due to the specific functional groups in quercetin were identified from the FTIR spectra of quercetin powder and nanoparticles. The infrared spectrum of quercetin powder also showed two peaks that could be attributed to O-H stretching which was observed at 3415 cm -1 while C=O and C=C stretching vibrations were observed at around 1654 cm -1 and 1600 cm -1 respectively. These peaks corresponded to the conventional flavonoid skeleton of quercetin. Hydroxyl groups were further identified by the C-O stretching vibrations which were attributed to the peaks at 1243 cm^{-1} and 1082 cm^{-1}. Similar peaks were observed in the FTIR spectrum of quercetin nanoparticles with differences in shift and intensity which indicates that there is a change of interaction in the molecule due to nanoparticle formation. For instance, the O-H stretching peak which occurred at about 3420 cm^{-3} was indicative of the fact that the formation of nanoparticles interfered with hydrogen bonding. The C=O stretching peak appeared at around 1650 cm^{-3}, whereby slight shift indicated that the stabilisers/surfactants used in preparation of the nanoparticles might have interacted with the quercetin molecule. As seen from the above assignment the small shift in the C-O stretching peaks to 1244 and 1084 cm^{-3} is an evidence that the hydroxyl groups surroundings have transformed due to the formulation of the nanoparticles. These alterations confirmed the successful synthesis of quercetin nanoparticles and also the usage of the functional groups in stabilizing the nanoparticles.

ANTIOXIDANT ACTIVITY OF QUERCETIN POWDER AND QUERCETIN NANOPARTICLES

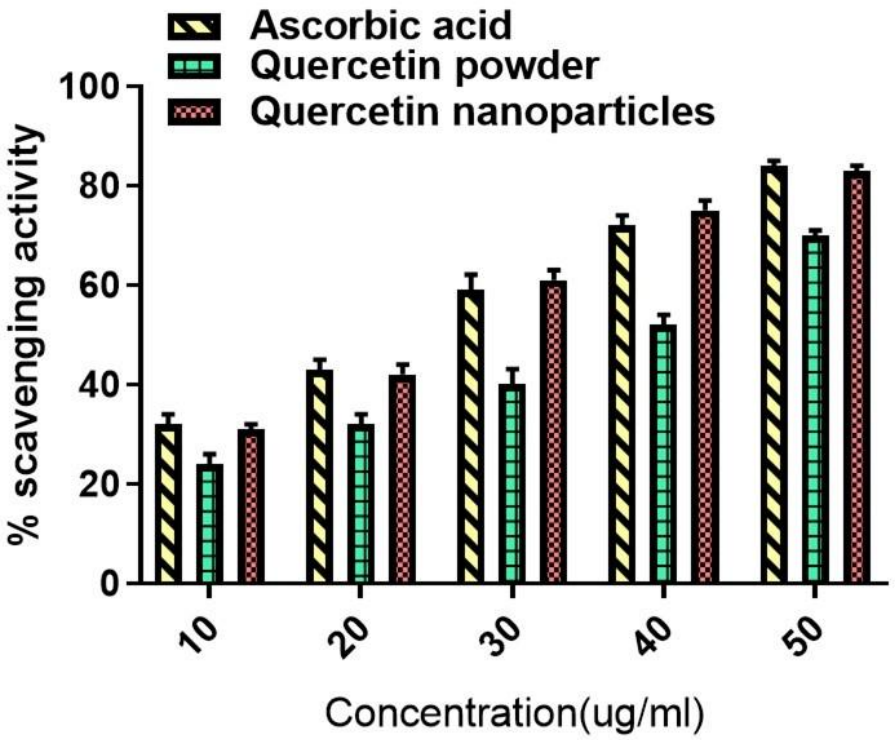

FIGURE 2:ANTIOXIDANT ACTIVITY OF QUERCETIN POWDER, QUERCETIN NANOPARTICLES AND STANDARD ASCORBIC ACID, QUERCETIN NANOPARTICLES SHOWS ANTIOXIDANT RESULTS VERY CLOSE TO STANDARD.

Antioxidant activity of quercetin powder and that of quercetin nanoparticles were determined at various concentrations (10, 20, 30, 40, and 50 µg/ml) and compared with ascorbic acid, a standard antioxidant.

Further it was observed that the antioxidant activity of ascorbic acid was 32% at 10 µg/ml and standard deviation was ±2. Compared to this, the quercetin powder was found only to have 24% of antioxidant activity with standard deviation of ±2 while quercetin nanoparticles were found to offer a 31% protection against ± 1. Antioxidant activity of ascorbic acid raised to 43 % (± 2) while the quercetin powder raised to 32 % (± 2),, and quercetin nanoparticles also raised to 42 % (± 2) as concentration reached 20 µg/ml. The ascorbic activity enhanced to 59% (±3. 1) for 30 µg/ml Q-powder and 40% (±3. 1) for Q-nanoparticles. In this and subsequent experiments ascorbic acid showed 72% (±2) activity at 40 µg/ml, Q-powder 52% (±2), Q-nanoparticles 75% (±2). From this study, it was evident that at the highest concentration of 50 µg/ml ascorbic acid had 84% (±1) of antioxidant activity Q-powder had 70% (±1) and Q-nanoparticles had 83% (±1) antioxidant activity. These findings show that Myrtus communis extract has great antioxidant activity and how its activity increases with concentration and is almost same as ascorbic acid at higher concentrations.

HEPATOPROTECTIVE (HEMOSTATIC) EFFECT OF QUERCETIN NANOPARTICLES IN RATS:

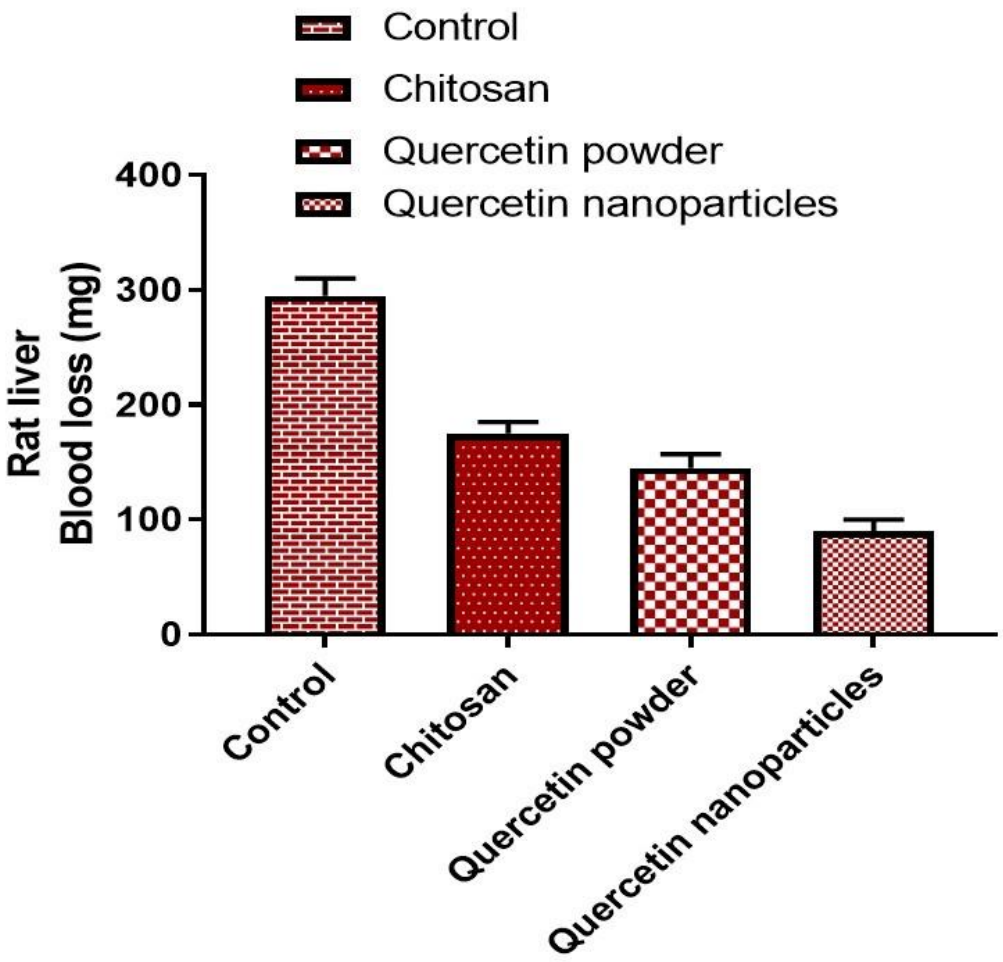

FIGURE 3:HEPATO PROTECTIVE EFFECT OF CONTROL, CHITOSAN, QUERCETIN POWDER AND QUERCETIN NANOPARTICLES

Quercetin powder and nanoparticles-treated group hepatoprotection was compared with chitosan (standard) and treated group. The control group revealed the average blood loss from liver to be 295±15 mg meaning that the patient's clot formation was slow. On the other hand, due to the active haemostatic effect of chitosan it was possible to shorten it to 175 mg with the variability of ±10. There was also a more emphasized effect of quercetin powder in the experiment where it reduced to 145 mg, while nanoparticles of Quercetin decreased it to 90mg ±12. From these findings it can be recommended that quercetin nanoparticles may be a potential natural agent that enhance blood clotting since its haemostatic effect appears to be higher than chitosan and the control.

BIOCHEMICAL INVESTIGATION OF QUERCETIN POWDER AND QUERCETIN NANOPARTICLES:

TABLE 1: EFFECTS OF Q-POWDER AND Q-NANOPARTICLES ON LIVER ENZYMES (ALT, AST AND ALP) IN RATS.

Groups	ALT (IU/L)	AST(IU/L)	ALP(IU/L)
Control	26.01±1	55±2.5	80±6.5
Q-powder	22±1	51±1.5	75±5.5
Q-nanoparticles	17±1	44±1	62±3.0

The results reveal how Q-powder and Q-nanoparticles alter the activities of liver enzymes in rats That include alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). In the control group the ALT level is 26. The result in the sham group is only slightly above the control groups but still falls within normal range at 01±1 IU/L. When Q-powder is given, the liver's ALT is lowered to 22±1 IU/L which shows moderate protection is provided by it. On the other hand, Q-nanoparticles are found to be reducing this parameter to a larger extent as the ALT decreases to 17 ± 1 IU/L further confirming the effect of Q-nanoparticles in preventing hepatic diseases. Similarly, for the control group the level of AST (55± 2. 5 IU/L) is highest to check the health of muscles and liver.

The hepatoprotective effects of the nanoparticle formulation are augmented as Q-powder also lowers AST to 51±1. It was 75±2 IU/L and Q-nanoparticles decrease it to 44±1 IU/L. There is no significant difference of ALP levels between the Q-powder group, control group and Q-nanoparticles group and they all have 80±6. 5 IU/L, 75±5. 5 IU/L, and 62±3. 0 IU/L, respectively. A more social effect in maintaining the ideal liver enzyme level is supported again by the lower ALP level amongst the group that was administered Q-nanoparticles. In cytoprotective effect, it was evident that, like Q-powder, Q-nanoparticles are also beneficial but the latter has a superior effect in protecting the liver by reducing the levels of liver enzymes more than Q-powder formulation.

HISTOPATHOLOGICAL EXAMINATIONS

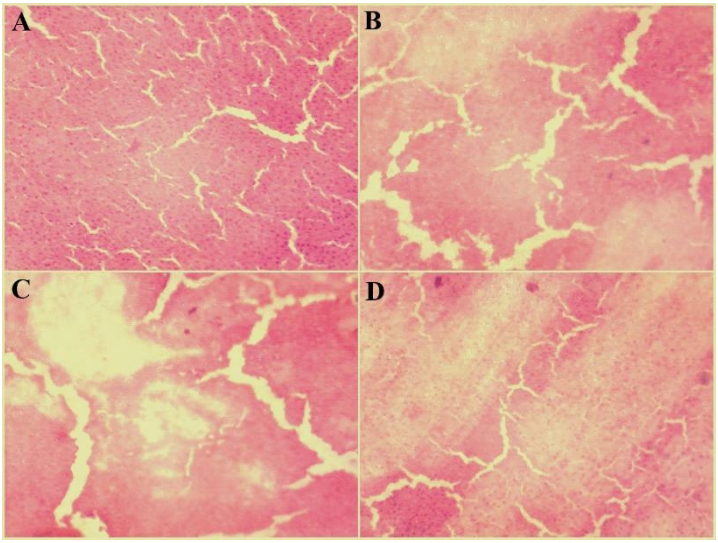

FIGURE 4:HISTOPATHOLOGY (A) NORMAL RAT LIVER (B) Q-POWDER EFFECT ON RAT LIVER (C) CCL4 INDUCTION (D) Q-NANOPARTICLES EFFECT ON RAT LIVER. AS ILLUSTRATED IN FIGURE C, THE LIVER OF RAT EXPOSED TO CCL_4 DISPLAYED SEVERE HEPATOTOXICITY EFFECTS SUCH AS NECROSIS, INFLAMMATIONS AND FATTY DEGENERATION. THIS IS EVIDENT FROM (B) AND (D) SHOWING THAT THE RAT THAT WAS ADMINISTERED WITH Q-POWDER AND Q-NANOPARTICLE HAS REGAINED MOST OF THE FAVOR DAMAGED BY CCL_4.

Histological examination of the rat liver presented in Fig. 4 elucidates marked dissimilarities of the liver tissue treated by quercetin powder and nanoparticles, as well as the liver that remains intact after CCl_4 exposure and the liver with moderate damage. The first picture presents normal arrangement of hepatocytes and absence of inflammatory or injured phenomena in normal rat liver. As illustrated in figure C, the liver of rat exposed to CCl_4 displayed severe hepatotoxicity effects such as necrosis, inflammations and fatty degeneration. This is evident from (B) and (D) showing that the rat that was administered with Q-powder and Q-nanoparticle has regained most of

the favor damaged by CCl_4. In this context, reduced inflammation and cell damage are detected, and a more or less distinguishable architectural recovery of the liver, evident of the hepatoprotective property of Q-nanoparticles. This one supports liver reformation and healing; thus, manages liver destruction resulting from CCl4.

DISCUSSION

From the FTIR spectra of the quercetin powder and quercetin nanoparticles, one can identify definite peaks that corresponds to the functional groups of the plant in question. The wide upsurge in quercetin powder at 3415 cm^{-1} suggests the presence of a hydroxyl group while the peaks at 1654 and 1600 cm^{-1} are as a result of carbonyl and olefinic vibrations of the flavonoid skeleton (Zhang et al., 2008). Moreover, the presence of hydroxyl group is further evidenced by the C-O stretching frequencies at 1243 cm^{-1} and at 1082 cm^{-1}. The FTIR spectrum reveals similar peaks which have slightly shifted and reduced their intensity when quercetin is incorporated in the nanoparticles, which suggests changes in the chemical interactions that occur during nanoparticle formation. The symmetric stretching of O–H band, for instance, arises at 3420 cm^{-3}, suggesting influence of hydrogen bonds. A change of the C=O stretching peak to 1650 cm^{-1} indicates that quercetin molecules have an interaction with stabilisers or surfactants used in the formation of nanoparticles. Similarly, the changes of the C-O stretching peaks to 1244 & 1084 cm^{-1} refer to the alterations in the vicinity of hydroxyl groups which, in turn, confirm the formation and stabilisation of nanoparticles (Kumari et al., 2010). Comparing ascorbic acid as standard to quercetin powder and quercetin nanoparticles, antioxidant activity was evaluated by incubating samples at 10, 20, 30, 40 and 50 µg/mL concentrations. This revealed that quercetin powder and quercetin nanoparticles delivered antioxidant activities of 24% and 31% at 10 µg/ml respectively as compared to 32% of ascorbic acid. It also favorably followed the concentration-response trend, meaning that antioxidant activity increased as concentration did. Quercetin nanoparticles exhibited 83% antioxidant activity at 50 µg/ml while quercetin powder exhibited only 70% and of compound was nearly equal to ascorbic acid i. e. 84%. From these results, it can be concluded that quercetin nanoparticles possess a greater potential for antioxidant activity compared with quercetin selling in the form of powder, particularly at a high concentration (Abid et al., 2022). This is most probably due to their larger surface area and better biologic availability. Haemostasis time with reference to chitosan and a control was applied in determining haemostatic properties of quercetin powder and nanoparticles. The haemostasis time in the control group amounted to 295 seconds, indicating that the clotting process was quite slow; by using chitosan that has been previously shown to possess haemostatic properties this time was reduced to 175 seconds. Most importantly absorption profile, quercetin nanoparticles and powder both reduced haemostasis time to 90sec and 145sec respectively. Based on this, quercetin specifically as nanoparticles enhances clotting efficiency much beyond the control and chitosan (Ubaid et al., 2025). This shows that nanoparticles result in better haemostatic effect of the anti-coagulant highlighting that this is a natural agent that has the potential to promote faster clotting of the blood. Further assessments of quercetin formulations on alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the liver were also performed. The ALT level in the control group was 26. range of about 01± of 1 IU/L and this is more or less within normal limits. Q-powder showed moderate anti-hepatotoxicity with normalization of ALT level to 22±1 IU/L then Q-nanoparticles presented higher hepatoprotective activity indicated by ALT level equal to 17±1 IU/L. Similarly, in control group the AST level reported at 55±2. 5 IU/L, which is in the range of possible liver stress. More potent hepatic preserving properties were depicted by Q-nanoparticles through lowering AST level to 44±1 IU/L from 51±1. Thus, they decreased from 10 IU/L by the action of Q-powder to 5 IU/L. The control group was observed to have the following ALP level; 80 ± 6. 5 IU/L, Q-powder's 75±5. 5 IU/L, and Q-nanoparticles' 62±3. All of them were not detected or were at very low levels, including 0 IU/L, in a similar fashion. Compared to Q-powder, Q-nanoparticles show much higher hepatic function preservation because of much stronger ability in balance of liver enzymes with less decline

in ALP. The hepatoprotective effects of quercetin formulations are further supported from histological changes. The liver treated with CCl_4 shows extensive hepatotoxicity characterized by necrosis, inflammation and fatty change while the normal liver shows well organized hepatocytes. Considerable improvement was observed in response to Q-powder and Q-nanoparticles therapy as far as inflammation reduction and liver tissue regeneration in the animals treated by Q-nanoparticles. The liver regeneration observed post treatment by Q-nanoparticles shows the potential of these particles in liver healing and protection from CCl_4 mediated damage. Altogether in the given work proves that the usage of quercetin nanoparticles contributes to higher antioxidant, hepatoprotective and haemostatic activity compared with quercetin powder(Miltonprabu et al., 2017).

CONCLUSION

By using a chemically-induced liver damage model, this study effectively showed that quercetin nanoparticles have greater hepatoprotective effects than quercetin powder. Because of QNPs' enhanced bioavailability, anti-inflammatory, antioxidant, and capacity to support liver regeneration, they represent a promising option for hepatoprotective treatment research. This study contributes to the increasing amount of evidence that supports the use of nanoparticles in drug delivery and creates new avenues for the development of more potent treatments for liver diseases and possibly other conditions that may benefit from the use of natural compounds like quercetin.

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