Development of the Hepatoprotective Therapeutic Binary Complex of Acetaminophen and Quercetin through Co-Crystallization Approach

Monisha K Madheswaran[1], SenthilRajan Dharmalingam[1], Geetha Srinivas[1] YashodaMariappa Hegde[1]

[1]Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Elayampalayam (P.O), Tiruchengode 637-205, Tamilnadu, India

Purpose: The present work aimed to create acetaminophen (APAP) co-crystals with QUE to improve the solubility of QUE and reduce Hepatotoxicity caused by APAP treatment. **Methods:** A slow solvent evaporation method was used to create APAP and QUE co-crystals, which were then, characterized using Fourier Transform Infrared spectroscopy (FTIR), Powder X-Ray Diffraction (PRXD), and Differential Scanning Calorimetry (DSC). In-vitro dissolution and solubility studies were also successful. Albino Swiss mice were used in an invivo single-dose hepatotoxicity investigation for produced co-crystals. Animals were given a single toxic dose of 300 mg/kg of APAP, as well as its dose equivalent in the physical mixture (PM) and co-crystals (CC). After 24 hours, serum liver indicators including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatases (ALP) were examined and compared to control and APAP-treated groups. **Results:** The outcomes of the characterization showed that the crystalline phase transformation had occurred, indicating the development of new solid co-crystalline structures. The permissible drug content for APAP (96.9858 1.4921 per cent) and QUE (89.2857 3.5714 per cent) was determined by UV-VIS analysis. With an 18.9-fold increase in aqueous solubility and a 10.38-fold increase in dissolution at 180 minutes, QUE has achieved exceptional solubility and dissolution profile. The liver enzyme biomarkers were reported to be similar in the control and co-crystals treated groups without showing significant differences $(p > 0.05)$, indicating that QUE effectively counteracted the HT of APAP and that it almost completely removed the HT due to the advantages of increased QUE solubility achieved by co-crystallizing with APAP. **Conclusion**: The study found that co-crystallization effectively increased the solubility of QUE and eliminated the HT associated with APAP, implying that APAP administration could be made safe and effective.

Keywords: Acetaminophen (APAP), Quercetin (QUE), Hepatotoxicity, Co-Crystals, Solubility, Slow Evaporation Method.

I. INTRODUCTION

Co-crystallization is a common technique used in the pharmaceutical industry to enhance the Active Pharmaceutical Ingredients (APIs). It is a novel and promising technique that tailors the physicochemical parameters of the active pharmaceutical moiety to produce a distinct crystalline structure through intermolecular interactions [1]. This solid-state customization approach enhances pharmaceutical characteristics such as solubility, dissolution, bioavailability, compressibility, and stability of active pharmaceutical ingredients (APIs) [2,3]. Pharmaceutical salts, polymorphs, hydrates, solvates, and novel co-crystals are among the crystal engineering techniques [4]. However, in order to optimize its physicochemical characteristics, the drug development process needs an investigation of the ideal solid form of API [5, 6].

There from, using an appropriate co-former, co-crystallization is a good choice for engineering a broad array of solid-state API forms [7]. Pharmaceutical co-crystal is a multi-component system in which the API interacts non-covalently with the co-former to produce homo/hetero molecular synthons [1]. Rather than formulating traditional solid-state forms such as salts and searching for different polymorphic forms, the formulation of co-crystals has proven to be a powerful strategy, with improved solubility, dissolution rate [8,9], stability [10], permeability[11], and compressibility [12] of API, resulting in increased bioavailability. Co-crystals

are also used to reduce drug-related adverse effects [13, 14].

Acetaminophen (APAP) is the most widely used analgesic and antipyretic in the world, and it is available as a mono or fixed-dose multi-combination on prescription or over-the-counter (OTC) globally [15]. In patients who are contraindicated to non-steroidal analgesics, pregnant women, breastfeeding women, and children with a fever associated with a disease, APAP is a medicine of choice [16]. It is suggested for use in both acute and chronic pain [17,18]. APAP is still the first-line therapy for mild to moderate pain in children and the elderly [19,20]. Hepatotoxicity (HT) and acute hepatic failure (ALF) are the most prevalent adverse effects associated with APAP use, despite its extensive therapeutic usage as an analgesic and antipyretic [16]. Because of the saturation of APAP glucuronidation and sulfonation, as well as the depletion of glutathione (GSH), a toxic metabolic product called N-acetyl-p-benzoquinone imine is produced (NAPQI). Furthermore, because toxic NAPQI and free radicals bind covalently with hepatic cell protein, hepatic cell damage can lead to HT and/or ALF [21,22,23].

Quercetin (QUE), a flavonol subclass of flavonoids abundantly found in tea, fruits, leaves, pod vegetables, and red wine [24], is a vital component of the human diet. It has been extensively studied for its antioxidant properties [25]. The oxidative effects of free radicals can be reduced by this flavonol with a polyphenolic backbone [26]. QUE reduces oxidative stress and the damage it causes by scavenging reactive oxygen and nitrogen species (ROS and RNS) [27]. QUE has been shown to have hepatoprotective properties against APAP, and its inhibitory action on cytochrome (CYP) 2E1 and other CYP mediated APAP biotransformation can decrease the production of NAPQI in previous investigations. It can reduce oxidative and nitrative damage while also lowering lipid peroxidation [28-31].

The production of NAPQI is an important process implicated in APAP-induced hepatic necrosis [28]. As a result, using QUE to reduce the progression of liver damage associated with APAP treatment might be a protective strategy. However, QUE's effectiveness is restricted due to its weak solubility and low bioavailability [32]. The pharmacological properties of both QUE and APAP may be worsened if taken together. Because of its poor solubility, QUE has to be at a high concentration to protect against APAP-induced

toxicity. As a result, it requires intelligence to create the optimum formulation to increase QUE solubility, which represents APAP administration safety. Our goal was to create a co-crystal of APAP utilizing QUE that had enhanced physicochemical characteristics due to parallel advantages. APAP is a high-aqueous-solubility BCS class III drug [33]. Co-crystallization with QUE increases its aqueous solubility, which improves its oral bioavailability and eventually feeds back to APAP to reduce its adverse effects. Three aromatic rings with five hydroxyl groups (-OH), an ether (-O-) moiety, and a carbonyl group (C=O) make up the QUE molecule. The five –OH groups are hydrogen bond donors (HBD) and acceptors (HBA) (HBA). Hydrogen bond acceptors are the ether and carbonyl moieties. As a result of QUE's facilitation of hydrogen bond formation/other interactions with APAP's phenolic and amide functional groups, supramolecular homosynthon (O...H-O) or heterosynthon (O...H-N) production occurs [34, 35]. As a result, APAP with QUE co-crystals was synthesized, described, and tested in order to see if it had a superior therapeutic impact.

Crystal structure can be critical to the performance of a dosage form. This is particularly evident for substances with inherent drug delivery barriers, such as low aqueous solubility, slow gastrointestinal dissolution, low permeability, and first-pass metabolism. Water insoluble compounds that must be administered orally in high dosages have the greatest impact on bioavailability characteristics due to the nature of their physical form and formulation. The application of crystal engineering of co-crystals is an alternate strategy accessible for improving drug solubility, dissolution, and bioavailability. Unlike salts, which require ionizable functional groups, co-crystallization can be used for compounds that lack ionizable functional groups. The physicochemical qualities of active pharmaceutical components, as well as the bulk material properties, can be altered while the intrinsic activity of drug molecules is maintained. The novelityof this research is to summarise the importance of crystal engineering in improving the physicochemical properties of a drug, such as in our study, where co-crystallization increased Quercetin solubility and eliminated hepatotoxicity associated with Acetaminophen, implying that Acetaminophen administration could be made safe and effective.

II. METHODS

2.1 Chemicals

APAP of pharmaceutical-grade was obtained from Farmson Pharmaceuticals Gujarat Private Limited, Gujarat, India. QUE and all other reagents used were reagent grade purchased from LobaChemie Private Limited, Mumbai, India.

2.2 Development of APAP -QUE co-crystals and physical mixture [36]

APAP (2 mM) and QUE (1 mM) co-crystals (CC) were made by mixing and dissolving in 75 mL of acetone. For 6 hours, the solution was swirled continuously at room temperature using a magnetic stirrer (MS 2012, Elecktrocraft Private Limited, Mumbai, India). The resultant solution was filtered and refrigerated overnight, after which the solvent was allowed to evaporate slowly and entirely at room temperature for another 24 hours. The fine APAP-QUE CC was collected separately and kept in an airtight container after sieving using a 100 mesh screen. For the developed co-crystals, the shape and melting point were evaluated.

By homogeneously mixing APAP (2 mM) and QUE (1 mM) in a glass mortar and pestle, a physical mixture (PM) of APAP and QUE was formed. For further evaluation, the APAP-QUE PM was collected, sieved through sieve no.100, and kept in an airtight container.

2.3 Characterization of APAP-QUE CC

2.3.1 Fourier transform infrared spectroscopy analysis

The infrared spectrum of APAP, QUE, and APAP-QUE CC was obtained using an FTIR-8400S spectrophotometer (Shimadzu Corporation, Japan). The sample pellets were produced on a KBr press under hydraulic pressure of 100kg/cm2 after being finely crushed with potassium bromide (KBr). The spectral wavenumber range was set between 4000 and 400 cm-1, and the spectrum was recorded after the samples were scanned The crystalline state of APAP, QUE, and manufactured co-crystals was examined using a Brucker D 8 Advance Eco-Powder X-ray diffractometer (Brucker Corporation, Germany). Cu Kα as the source radiation (1.5406A) and the Lynxeye detector in symmetrical reflection mode was used for PXRD investigations. Standard runs were carried out in this technique at 40 mA and 40 kV. The samples were scanned at a rate of 0.2 sec/min in the scanning 2 range of 10 to 80. The amount of reflected radiation was measured.

2.3.2 Differential scanning calorimetry analysis

DSC 50 (Shimadzu Corporation, Japan) was used to conduct thermal analysis on APAP, QUE, and co-crystals. The samples were weighed, placed in metal pans, and sealed thematically. The samples were run in a nitrogen gas environment with a nitrogen flow rate of 40 mL/min, a heating rate of 10 degrees Celsius per minute, and a temperature range of 30 to 300 degrees Celsius.

2.4 Evaluation of APAP-QUE CC

2.4.1 UV-Vis Spectroscopic analysis

In a sufficient volume of methanol, the calculated amount of produced co-crystals corresponding to 10.003 mg of APAP and 20 mg of QUE was dissolved and diluted. A 2203 smart UV-Vis spectrophotometer (Systronics India Limited, Gujarat, India) was used to determine the quantity of drug present in co-crystals at λ max of 244.8 nm for APAP and 377.6 nm for QUE in co-crystals. The drug content analysis was done in triplicate.

2.4.2 Solubility analysis [36]

The solubility of APAP, QUE, and APAP-QUE CC in distilled water was analyzed. The experiment involved dissolving 500.03 mg, 1000 mg, and 1500 mg of APA, QUE, and co-crystals in 100 mL of solvent, respectively. The mixtures were shaken continuously at 150 rpm for 48 hours at room temperature in a mechanical shaker (Orbitek LT-Orbital, Scigenics Biotech Pvt Ltd, Chennai, India). Finally, each sample's solution was filtered, and a sufficient dilution with filtrate was prepared to determine solubility. Using a UV-Visible spectrometer set to 374.4 nm for QUE and 254.4 nm for APAP, the concentration in co-crystals of the above-diluted sample solutions was determined. The solubility of the APIs was found at 244.8 nm for APAP and 377.6 nm for QUE, respectively. Each sample's solubility analysis was carried out in triplicate.

2.4.3 In-vitro dissolution studies [37]

The in-vitro dissolution was carried out using the USP type II paddle technique in the VDA-8D dissolution equipment (Veego Instruments Corporation, Mumbai, India). Throughout the in-vitro dissolution testing of standard API and co-crystal formulation, the rotation speed was kept at 100 rpm and the bath temperature was kept at 370.5°C. Dissolution experiments were performed on the standard API and a sample of 75 mg of co-crystals corresponding to 25.007 mg of APAP and 50 mg of QUE. As a

dissolving media, phosphate buffer pH (7.4) was employed (900 mL).

APAP and QUE

At certain predefined time intervals, 1 mL samples were taken (0, 5, 10, 15, 30, 45, 60, 90, 105, 120, 150, and 180 min). The sample was replaced with 1 mL of fresh media after each withdrawal. After appropriate dilution, all samples were spectrophotometrically examined at 244.8 nm and 377.6 nm for APAP and QUE, respectively, to determine the proportion of drug dissolved. The release study was carried out in triplicate.

APAP-QUE CC

At certain predefined time intervals, 2 mL samples were taken (0, 5, 10, 15, 30, 45, 60, 90, 105, 120, 150, and 180 min). The sample was replaced with 1 mL of fresh media after each withdrawal. After appropriate dilution, all samples were spectrophotometrically examined at 254.4 nm and 374.4 nm for APAP and QUE, respectively, to determine the percentage of drug dissolved. The release study was carried out in triplicate.

2.5 Single-dose hepatotoxicity study

During this investigation, the animals were treated humanely in accordance with Indian National Science Academy norms, and the experiments were carried out according to CPCSEA rules. In this investigation, healthy adult Swiss albino mice of either sex (20-25 g) were utilised. They were maintained in polypropylene cages in an air-conditioned animal house with plenty of ventilation. The normal laboratory conditions were maintained, with a 12 hour light/dark cycle, a temperature of (25±2°C), and humidity of (50±15%). The animals were obtained from Swamy Vivekanandha College of Pharmacy's central animal house. The animals were fed ad libitum a standard pellet diet and water. Twenty-four mice weighing 20-25 g were randomly split into four groups of six mice each (control untreated group, APAP treated, APAP-QUE CC treated, and APAP-QUE PM treated). Blood samples were collected through the retro-orbital plexus at 24 hours after animals were given co-crystals and physical mixture (PM) in a dose equivalent to 300 mg/kg of APAP and plain APAP in a dose equivalent to 300 mg/kg of APAP to corresponding animal groups, and serum was separated and estimated for alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). The institutional animal ethics committee accepted the animal study protocol [13, 36, 38, 39, 40].

Table 1- A summary of treatment groups, dose and animals required for study

S. No	Treatment Groups	Dose	Animals Required
1	Group I Normal control	Vehicle (vegetable oil) p.o. at 8th day	6
2	Group II APAP treated	300mg/kg, p.o. on 8th day	6
3	Group III APAP-QUE CC	Treated with 90 mg/kg p.o. on 8th day (dose equivalent 300 mg/kg of APAP)	6
4	Group IV APAP-QUE PM	Treated with 900 mg/kg p.o. at 8th day (dose equivalent 300 mg/kg of APAP)	6

2.5.1 Parameters to be evaluated [24]

After administration of co-crystals and the physical mixture (PM) in a dose equivalent to 300mg/kg of APAP to animals and plain APAP of about 300mg/kg, the blood samples were collected through a Retro-orbital plexus at 24 hour, and the serum will be separated and analyzed for the further biochemical estimation.

1. Estimation of Alanine transaminase (ALT/SGPT) (IU/L)
2. Estimation of Aspartate transaminase (AST/SGOT) (IU/L)
3. Estimation of alkaline phosphatase (ALP) (IU/L)

2.6 Statistical analysis

For solubility, dissolution, and in vivo HT study, one-way ANOVA followed by Dunnett test was used with Graph Pad Prism 5.0 software to discover the statistically significant difference between co-crystal and API.

III. RESULTS

APAP co-crystals were successfully produced with QUE utilizing the slow solvent evaporation method. When employing acetone as a solvent, needle-shaped co-crystals were the most common result. When comparing APAP and QUE, a microscopic examination of co-crystals revealed (Figure 1) that APAP-QUE CC produced long and wide yellow needle-shaped crystals. The melting temperatures of APIs were determined to be 167.15 ± 0.581 ○C (APAP) and 309.15 ± 0.243 ○C (QUE), respectively, while the melting point of co-crystals was calculated to be 258.74 ± 1.758 ○C.
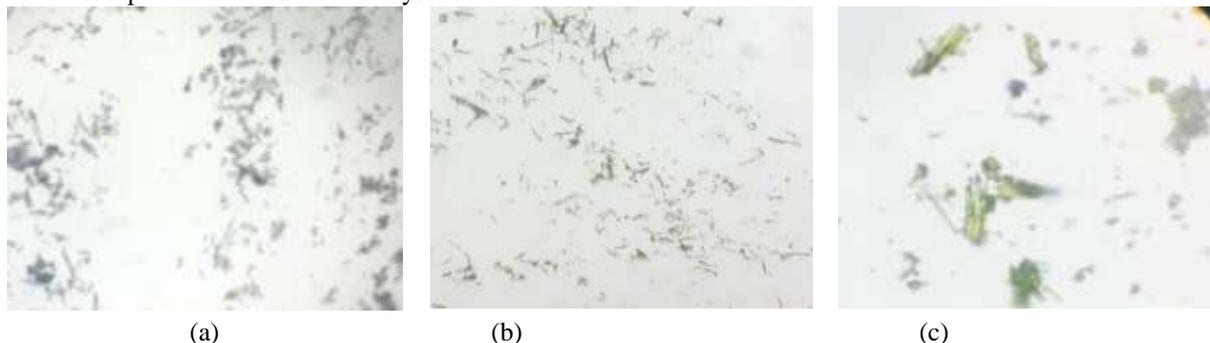

| (a) | (b) | (c) |

Figure: 1- Microscopic photograph of (a) APAP (b) QUE and (c) APAP-QUE CC

3.1 Characterization of APAP-QUE CC
3.1.1 Fourier transform infrared spectroscopic studies

The FTIR spectra of APAP, has shown peaks for N-H stretching and O-H stretching at 3326.01 cm-1 and 3110.00 cm-1, respectively. In QUE, the O-H groups stretching at 3406.05 cm-1 and 3288.40 cm-1 were discovered. The literature data corroborated the observed FT-IR results of APAP and QUE. [41, 42] The initial components of the co-crystal spectrum were discovered to be different. It revealed a shift in the peaks that corresponded to it (3326.01 to 3325.05 cm-1, 3110 to 3115.40 cm-1 and 3406.05 to 3398.34 cm-1 and 3288.40 to 3298.34 cm-1).

1.1.2 Powder X-ray Diffraction analysis

Figure 2(a) and 2(b) show the powder X-ray patterns of APAP and QUE (b) The QUE characterisation peak was detected at various diffraction angles of 10.695 ○ , 12.391 ○, 15.783 ○, 24.325 ○, 26.511○, and 27.245 ○ [43], whereas the APAP characterization peak was observed at 12.084 ○, 15.456 ○, 18.03 ○, 20.381 ○, 23.405 ○, 24.325 ○, and 26.429 ○, which was verified by existing literature data. New co-crystal features peaks were observed at 11.70 ○, 13.78 ○, and 17.85○ [44], as shown in the figure: 2 (c). In the diffraction pattern of APAP-QUE CC, there was also a shift in 2 values at 15.4765 ○, 20.28 ○, 23.55 ○, 24.57 ○, and 26.53 ○, which differed from the corresponding beginning component. However, the co-crystals diffraction pattern revealed that 12.08 ○, 18.03 ○, 16.66 ○ of APAP and 10.69 ○, 12.39 ○ of QUE were vanished.
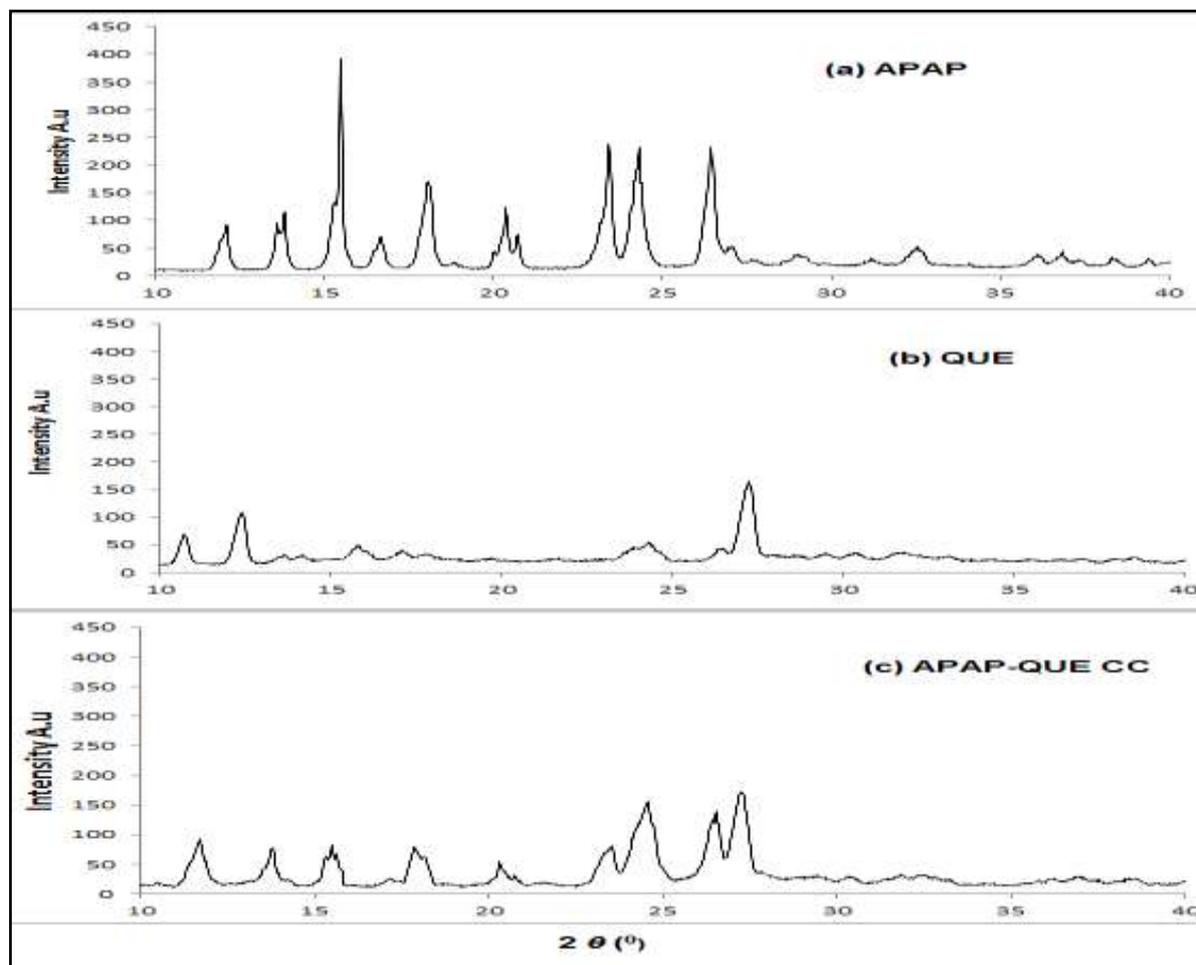
Figure: 2- Powder X-ray DiffractionPattern

3.1.3 Differential Scanning Calorimeter analysis

The APAP endotherm curve has a strong peak at 170.5 ̊C, QUE's two endothermic events which occurred at 318.7 ̊C and approximately 110 ̊C. The DSC pattern of the resultant co-crystals exhibited an endothermic peak at 277.8 ̊C.

3.2 Evaluation of APAP-QUE CC
3.2.1 UV-VIS spectroscopic analysis

The drug content examination was done in triplicate, and the drug concentrations in the developed co-crystals were found to be adequate,

with 94.499 ± 2.2792 %(APAP) and 89.2857 ± 3.5714 % (APAP) respectively (QUE).

3.2.2 Solubility analysis

In an Orbitek shaker, the solubility was measured three times in distilled water. The aqueous solubility of QUE, QUE in CC, and PM were computed and shown in table 1: exhibited improved co-crystallization. Table 1 also included APAP's aqueous solubility data. When comparing APAP to co-crystals, APAP solubility increased, while PM solubility remained the same.

Table 2 - Solubility data of APAP and QUE in raw, CC, and PM.

Solubility	Raw component	APAP-QUE CC	APAP-QUE PM
QUE	0.0022 ± 0.00007	0.0416 ± 0.0028	0.0035 ± 0.0007
APAP	0.1404 ± 0.0003	0.1436 ± 0.0004	0.1418 ± 0.0025

3.2.3 In vitro dissolution study:

In vitro drug release experiments were performed on the APIs and co-crystals of APAP and QUE using the USP dissolution type II (paddle) technique. For QUE, APAP, APAP-QUE PM, and APAP-QUE CC, dissolution were done in triplicate, and percentage drug release was determined by measuring absorbance at 254.4 nm and 377.4 nm. The dissolution profile of QUE is shown in Figure 3. At 180 minutes, 89.14 % of QUE was freed from co-crystals, whereas around 8.85 % of QUE was dissolved. PM (10.32%) hasn't shown much difference in drug dissolution when it comes to QUE. The dissolution profile of APAP is seen in Figure 3. In the dissolving medium, the percentage of APAP dissolved from co-crystals (96.15%) and physical mixture (90.25%) showed no significant difference when compared to APAP (94.0553 %).
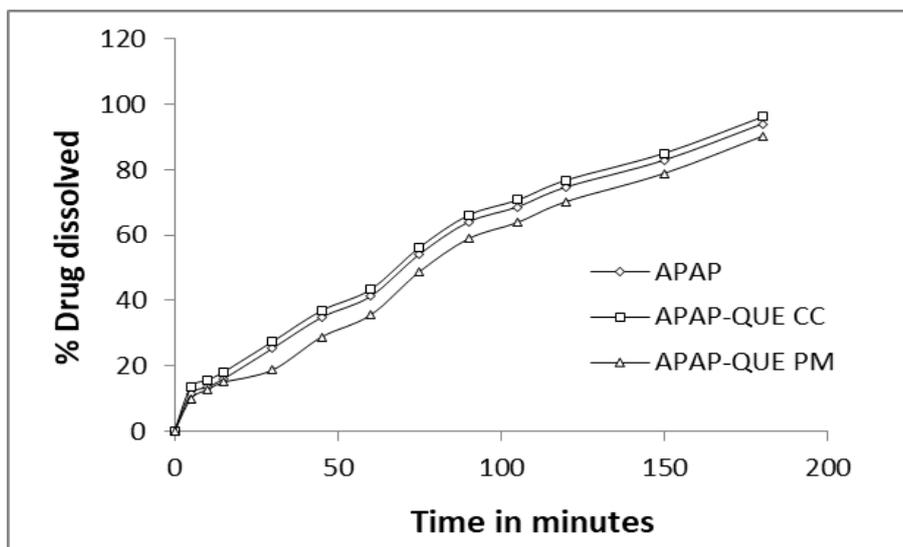

Figure: 3- Comparative Dissolution profile of APAP-QUE CC

3.2.4 Invivo Hepatotoxicity studies:

Single-dose in vivo hepatotoxicity experiments in mice were used to investigate the hepatoprotective effect of produced cocrystals. Figures 4(a), (b), and (c) show the levels of hepatic biomarkers measured in serum of each animal group (c). When APAP-treated mice were compared to normal control animals, hepatic serum indicators was found to be increased by 1.56 times for AST, 2.01 fold for ALT, and 1.49 fold for ALP. Normal vs CC and PM, APAP treated vs CC and PM, and CC vs PM have all been compared. When APAP-QUE CC was compared to the control group, it demonstrated a substantial difference in hepatic biomarker levels when they were made to correspond with APAP treated. The AST, ALP, and AST levels can be considerably reduced in both the APAP-QUE CC and APAP-QUE PM treated groups. When compared to cocrystals, the enzyme levels in APAP-QUE PM treated mice were lowered by 69.65% in AST, 71.33 % in ALT, and 77.87 % in ALP.
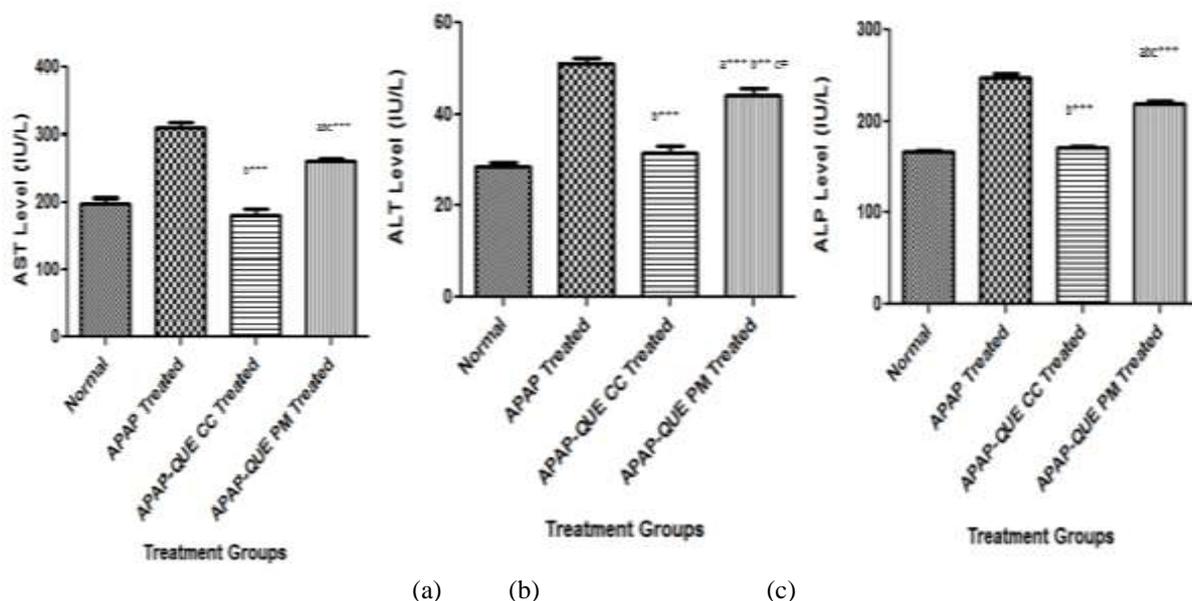
(a)   (b)   (c)

Figure: 4(a)- Comparision of level of AST in treatment groups, **(b)-** Comparision of level of ALT in treatment groups, **(c)-** Comparision of level of ALP in treatment groups.

The data is shown as Mean ± SEM (n=6). A comparison was done between the following groups: a) untreated Vs CC treated and PM treated, b) APAP treated Vs CC treated and PM treated, and c) CC treated Vs PM treated. The statistical significance was summarised using the symbols (***) = P<0.0001, (**) = P<0.001, and (#) P=0.0001.

IV.   DISCUSSIONS

By co-crystallizing APAP and QUE into a single crystal lattice, this research work has been designed to solve the related shortcomings with both. Because APAP and QUE molecules have hydrogen bond facilitated sites, the possibility and probability of co-crystal formation were evaluated using supramolecular methods before the co-crystals were developed. The co-crystals were then produced effectively. The APAP-QUE CC crystal shape was distinct from both APAP and QUE, implying the development of a novel co-crystalline structure. Due to the unification of two molecules on its crystal lattice, the observed co-crystals were found to be longer and wider.

Before going on to additional solid-state characterization, the melting point analysis was used to identify the co-crystal formation. Melting point determination is the most basic physical attribute used to identify and characterize a component's purity. It is just the point at which a solid transforms into a liquid [45]. The melting point of co-crystals differed from that of the corresponding starting API components. The melting point of co-crystals is similar to that of APIs. The creation of new crystalline solids might be attributed to a shift in the intermolecular configurations in the crystal lattice of APAP-QUE co-crystals [46].

The infrared absorption spectrums generated by Fourier transform infrared spectroscopy aids in the identification of chemical bonds in a molecule. Spectrum generates a molecule's unique molecular fingerprint, which may be used to filter, identify, and scan samples for constituents. There was considerable displacement and modification of the distinctive peaks in the FTIR spectra corresponding to APIs in the case of produced co-crystals. The development of non-covalent interaction bonds between APAP and QUE in co-crystals caused these modifications. Because the shifting was detected in the N-H amide and O-H stretching areas of APAP and the –OH stretching regions of quercetin, these non-covalent interactions might be caused by intermolecular hydrogen bonds. Hydrogen bonding with other hydrogen bonding functional derivatives is facilitated by this amide group and phenolic –OH (-OH in QUE).

PXRD is an analytical technique that is mostly used to determine the crystalline phase of a

solid. The non-covalent intermolecular interaction induced co-crystallization of API with co-former, resulting in a novel crystalline solid that can be characterised using PXRD. The emerged peaks in the diffractograms of APAP-QUE CC were recognised, which were previously not observed in the diffraction patterns of both APAP and QUE. When compared to co-crystals (less sharp), the diffractograms of APAP and QUE seemed to be sharper and more intense, and their distinctive peaks were also found to be missing in the co-crystals pattern, implying the presence of interaction. Finally, it was evident that the crystal lattice had changed, as co-crystals displayed a distinct PXRD pattern, indicating the development of a new solid co-crystalline phase.

Although melting point determination was used to determine the melting point of the APIs and co-crystals, DSC is a more complete analytical approach that is used for assessing the thermal behaviour of the components. QUE's thermogram showed 318.70°C, which is its melting point, and was also around 1100°C, indicating that it was dehydrating (QUE hydrate). The melting temperature of the peak identified in the DSC graph of co-crystals was between the melting temperatures of two APIs, suggesting the development of a new crystalline solid phase. It was found that more than half of the generated co-crystals had a melting point that was between the original API and the co-former[45].

To investigate the influence of co-crystallization on solubility, the aqueous solubility of QUE and APAP was investigated. When QUE was compared to QUE, its aqueous solubility in co-crystals was 18.9 times higher (P 0.001). PM also increased QUE solubility marginally, but not significantly (p > 0.05) compared to co-crystals. This demonstrates that there is no interaction in PM, but the presence of CC in non-covalent contacts aided solubility. The co-crystal formation can provide a crystal lattice with lower energy than pure APIs, perhaps increasing affinity for the solvent and bringing solubility [47]. In the case of APAP, co-crystals only exhibited a small increase in solubility, even in their co-crystalline condition, and no change in APAP solubility data in PM.Because solubility and dissolution are linked, the dissolution profile yielded comparable findings. At 180 minutes, both APAP and QUE in co-crystals were entirely dissolved. When QUE dissolution from co-crystals was compared to its raw form, it exhibited a 10.38 times increase in dissolution. The dissolution pattern of QUE from the physical combination was thought to be similar to the dissolution pattern of QUE alone, with no discernible differences between the two profiles. APAP-QUE co-crystals, on the other hand, experienced a more gratifying dissolution than QUE, which might be attributed to a decrease in free energy associated with the co-crystal solubilization process and the development of a metastable crystalline state on contact with the dissolving media. At 180 minutes, the dissolving curve revealed that 89.14 per cent of QUE was liberated from co-crystals, with a significant difference of p0.0001. However, there was no significant difference in the proportion of APAP dissolved from co-crystals and physical mixture in the dissolving media when compared to APAP (p>0.05).

The hepatoprotection of QUE against APAP and their co-crystallization effect were discovered in an invivo hepatotoxicity (HT) research, which demonstrated the hepatoprotection of QUE against APAP and their co-crystallization impact. The level of liver enzymes was higher in the APAP-treated groups. A single hazardous dosage of APAP (300mg/kg of mice body weight) can cause hepatic cell necrosis [39]. When compared to APAP-treated groups, APAP-QUE CC and APAP-QUE PM exhibited a sufficient positive degree of significance. PM decreased the liver enzyme level, which had been increased by APAP, due to the antioxidant nature of QUE. The physical combination-treated group, on the other hand, demonstrated less hepatoprotection than the co-crystals treated group, with a higher degree of decrease in liver serum biomarkers in the co-crystals treated groups. This discrepancy is due to QUE's low bioavailability. In the case of APAP-QUE CC, co-crystallization improved the solubility of QUE, potentially increasing its bioavailability. As a result, APAP-QUE co-crystals were reported to be efficacious against APAP-associated HT, potentially improving the safety and effectiveness of APAP treatment.

V.   CONCLUSIONS

The goal of this research was to develop better acetaminophen (APAP) dosage form for safe administration. As a result, we created APAP co-crystals with quercetin (QUE), an antioxidant flavonoid, to combat APAP hepatotoxicity (HT). The development of a novel crystalline phase was seen in the successfully produced APAP-QUE CC. The solubility and dissolution of co-crystals indicated that QUE had a very high solubility. In

vivo investigations revealed that APAP-QUE CC had a superior anti-HT effect. When compared to its physical mixture, this method enhanced the solubility of QUE, which may effectively counteract the HT of APAP. The benefit of complementing each other (APAP and QUE) in this binary system demonstrates the ingenuity of co-crystals in overcoming the toxic effect of API.

Abbreviations
APAP- Acetaminophen, **QUE**- Quercetin, **HT**-Hepatotoxicity, **PM**- Physical Mixture, CC- Co-crystals, **CPCSEA**- Committee For The Purpose Of Control And Supervision Of Experiments On Animals, **APAP-QUE CC**- Acetamiophen Quercetin Co-Crystals, **APAP-QUE PM**-Acetaminophen Quercetin Physical Mixture, **API**-Active Pharmaceutical Ingredients, **OTC-** Over The Counter, **NSAID**- Non-Steroidal Anti-Inflammatory Drug, **WHO**- World Health Organization, **GSH**- Glutathion, **APQI**- n-acetyl-p-benzoquinone imine, **ALF**- acute liver failure, **NAS**- New Active Substances, **USFDA-** United States Food And Drug Administration, **HBD-**Hydrogen Bond Donor, **HBA**- Hydrogen Bond Acceptor, **COX-**Cycloxygenase, **GIT**-Gastrointestinal Tract, **ALT-** Alanine Aminotransferase, **AST-**Aspartate Aminotransferase, **ALP-** Alkaline Phosphatase, **CYP**- Cytochrome, **ROS-** reactive oxygen species, **RNS**- Reactive Nitrogen Species, **DSC**-Differential Scanning Colorimetry, **FT-IR-** Fourier Transform Infrared Spectroscopy, **PXRD**- Powder X-Ray Diffractrometry, **RH-** Relative Humidity, **ASD**-Amorphous Solid Dispersions, **LD**- lethal dose, **FWHM**- Full Width At Half Maxima, **UV**-Ultraviolet, **SEM**- Standard Error Of The Mean

Conflict of Interest
The authors declare that they have no conflict of interest.

REFERENCES

[1] Sathisaran I, Dalvi SV. Engineering cocrystals of poorly water-soluble drugs to enhance dissolution in aqueous medium. Pharmaceutics. 2018 Sep;10(3):108. DOI: https://doi.org/10.3390/pharmaceutics10030108

[2] Varshosaz J, Ghassami E, Ahmadipour S. Crystal engineering for enhanced solubility and bioavailability of poorly soluble drugs. Current Pharmaceutical Design. 2018 Jun 1;24(21):2473-96.DOI: https://doi.org/10.2174/1381612824666180712104447

[3] Chattoraj S, Sun CC. Crystal and particle engineering strategies for improving powder compression and flow properties to enable continuous tablet manufacturing by direct compression. Journal of Pharmaceutical Sciences. 2018 Apr 1;107(4):968-74. DOI: https://doi.org/10.1016/j.xphs.2017.11.023

[4] Khadka P, Ro J, Kim H, Kim I, Kim JT, Kim H, Cho JM, Yun G, Lee J. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian journal of pharmaceutical sciences. 2014 Dec 1;9(6):304-16. DOI: https://doi.org/10.1016/j.ajps.2014.05.005

[5] Stahly P. Cocrystals: A Regulatory Rebirth. Whitepaper, Triclinic Labs. 2016.https://tricliniclabs.com/page-directory/publications-white-papers-tech-notes-bibliographies-from-the-scientists-at-triclinic-labs.html

[6] Zaini E, Afriyani A, Fitriani L, Ismed F, Horikawa A, Uekusa H. Improved Solubility and Dissolution Rates in Novel Multicomponent Crystals of Piperine with Succinic Acid. ScientiaPharmaceutica. 2020 Jun;88(2):21.DOI: https://doi.org/10.3390/scipharm88020021

[7] Abramov YA, Loschen C, Klamt A. Rational coformer or solvent selection for pharmaceutical cocrystallization or desolvation. Journal of pharmaceutical sciences. 2012 Oct 1;101(10):3687-97.DOI: https://doi.org/10.1002/jps.23227

[8] Panzade P, Shendarkar G, Shaikh S, Rathi PB. Pharmaceutical Cocrystal of Piroxicam: Design, formulation and evaluation. Advanced pharmaceutical bulletin. 2017 Sep;7(3):399.DOI:

https://dx.doi.org/10.15171%2Fapb.2017.04
8

[9] El-Gizawy SA, Osman MA, Arafa MF, El Maghraby GM. Aerosil as a novel co-crystal co-former for improving the dissolution rate of hydrochlorothiazide. International journal of pharmaceutics. 2015 Jan 30;478(2):773-8.DOI:https://doi.org/10.1016/j.ijpharm.2014.12.037

[10] Ober CA, Gupta RB. Formation of itraconazole–succinic acid cocrystals by gas antisolventcocrystallization. AapsPharmscitech. 2012 Dec 1;13(4):1396-406.DOI:https://doi.org/10.1208/s12249-012-9866-4

[11] Machado TC, Gelain AB, Rosa J, Cardoso SG, Caon T. Cocrystallization as a novel approach to enhance the transdermal administration of meloxicam. European Journal of Pharmaceutical Sciences. 2018 Oct 15;123:184-90.DOI: https://doi.org/10.1016/j.ejps.2018.07.038

[12] Persson AS, Ahmed H, Velaga S, Alderborn G. Powder Compression Properties of Paracetamol, Paracetamol Hydrochloride, and Paracetamol Cocrystals and Coformers. Journal of pharmaceutical sciences. 2018 Jul 1;107(7):1920-7.DOI:https://doi.org/10.1016/j.xphs.2018.03.020

[13] Yadav B, Gunnam A, Thipparaboina R, Nangia AK, Shastri NR. Hepatoprotective Cocrystals of Isoniazid: Synthesis, Solid State Characterization, and Hepatotoxicity Studies. Crystal Growth & Design. 2019 Jul 25;19(9):5161-72.DOI: https://doi.org/10.1021/acs.cgd.9b00541

[14] Yadav B, Balasubramanian S, Chavan RB, Thipparaboina R, Naidu VG, Shastri NR. Hepatoprotective Cocrystals and Salts of Riluzole: Prediction, Synthesis, Solid State Characterization, and Evaluation. Crystal Growth & Design. 2018 Feb 7;18(2):1047-61.DOI: https://doi.org/10.1021/acs.cgd.7b01514

[15] Ghanem CI, Pérez MJ, Manautou JE, Mottino AD. Acetaminophen from liver to brain: new insights into drug pharmacological action and toxicity. Pharmacological research. 2016 Jul 1;109:119-31.DOI: https://doi.org/10.1016/j.phrs.2016.02.020

[16] Tittarelli R, Pellegrini M, Scarpellini MG, Marinelli E, Bruti V, Di Luca NM, Busardo FP, Zaami S. Hepatotoxicity of paracetamol and related fatalities. Eur Rev Med Pharmacol Sci. 2017 Mar 1;21(1 Suppl):95-101.https://www.researchgate.net/publication/316283890_Hepatotoxicity_of_paracetamol_and_related_fatalities.

[17] Conaghan PG, Arden N, Avouac B, Migliore A, Rizzoli R. Safety of paracetamol in osteoarthritis: what does the literature say?. Drugs & aging. 2019 Apr 1;36(1):7-14.DOI: https://doi.org/10.1007/s40266-019-00658-9

[18] Cooper TE, Fisher E, Anderson B, Wilkinson NM, Williams DG, Eccleston C. Paracetamol (acetaminophen) for chronic non‑cancer pain in children and adolescents. Cochrane Database of Systematic Reviews. 2017(8). DOI: 10.1002/14651858.CD012539.pub2.

[19] De Martino M, Chiarugi A. Recent advances in pediatric use of oral paracetamol in fever and pain management. Pain and therapy. 2015 Dec 1;4(2):149-68.DOI: https://doi.org/10.1007/s40122-015-0040-z

[20] Makris UE, Abrams RC, Gurland B, Reid MC. Management of persistent pain in the older patient: a clinical review. Jama. 2014 Aug 27;312(8):825-37.10.1001/jama.2014.9405.

[21] Hinson JA, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. Adverse drug reactions. 2010:369-405. DOI: 10.1007/978-3-642-00663-0_12.

[22] Hodgman MJ, Garrard AR. A review of acetaminophen poisoning. Critical care clinics. 2012 Oct 1;28(4):499-516.DOI: https://doi.org/10.1016/j.ccc.2012.07.006

[23] Alipour M, Buonocore C, Omri A, Szabo M, Pucaj K, Suntres ZE. Therapeutic effect of liposomal-N-acetylcysteine against acetaminophen-induced hepatotoxicity. Journal of drug targeting. 2013 May 1;21(5):466-73.DOI: https://doi.org/10.3109/1061186X.2013.765443

[24] Miltonprabu S, Tomczyk M, Skalicka-Woźniak K, Rastrelli L, Daglia M, Nabavi SF, Alavian SM, Nabavi SM. Hepatoprotective effect of quercetin: From chemistry to medicine. Food and Chemical Toxicology. 2017 Oct 1;108:365-74.DOI: https://doi.org/10.1016/j.fct.2016.08.034

[25] Gupta C, Vikram A, Tripathi DN, Ramarao P, Jena GB. Antioxidant and antimutagenic effect of quercetin against DEN induced

hepatotoxicity in rat. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2010 Jan;24(1):119-28.DOI: https://doi.org/10.1002/ptr.2883

[26] Zhang M, Swarts SG, Yin L, Liu C, Tian Y, Cao Y, Swarts M, Yang S, Zhang SB, Zhang K, Ju S. Antioxidant properties of quercetin. InOxygen transport to tissue XXXII 2011 (pp. 283-289). Springer, Boston, MA. DOI: 10.1007/978-1-4419-7756-4_38.

[27] Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. European journal of pharmacology. 2008 May 13;585(2-3):325-37.DOI: https://doi.org/10.1016/j.ejphar.2008.03.008

[28] Pingili R, Pawar AK, Challa SR. Quercetin reduced the formation of N‑acetyl‑p‑benzoquinoneimine, a toxic metabolite of paracetamol in rats and isolated rat hepatocytes. Phytotherapy Research. 2019 Jul;33(7):1770-83.DOI: https://doi.org/10.1002/ptr.6365

[29] Barros PP, Silva GH, Gonçalves GM, Oliveira JC, Pagnan LG, Arco-e-Flexa L. Hepatoprotective effect of quercetinpretreatment against paracetamol-induced liver damage and partial hepatectomy in rats. Brazilian Archives of Biology and Technology. 2017;60.DOI: http://dx.doi.org/10.1590/1678-4324-2017160138

[30] El-Shafey MM, Abd-Allah GM, Mohamadin AM, Harisa GI, Mariee AD. Quercetin protects against acetaminophen-induced hepatorenal toxicity by reducing reactive oxygen and nitrogen species. Pathophysiology. 2015 Mar 1;22(1):49-55.DOI: https://doi.org/10.1016/j.pathophys.2014.12.002

[31] Tzankova V, Aluani D, Kondeva-Burdina M, Yordanov Y, Odzhakov F, Apostolov A, Yoncheva K. Hepatoprotective and antioxidant activity of quercetin loaded chitosan/alginate particles in vitro and in vivo in a model of paracetamol-induced toxicity. Biomedicine & pharmacotherapy. 2017 Aug 1;92:569-79.DOI: https://doi.org/10.1016/j.biopha.2017.05.008

[32] Ulusoy HG, Sanlier N. A minireview of quercetin: from its metabolism to possible mechanisms of its biological activities. Critical reviews in food science and nutrition. 2020 Oct 27;60(19):3290-303.DOI: https://doi.org/10.1080/10408398.2019.1683810

[33] Kalantzi L, Reppas C, Dressman JB, Amidon GL, Junginger HE, Midha KK, Shah VP, Stavchansky SA, Barends DM. Biowaiver monographs for immediate release solid oral dosage forms: Acetaminophen (paracetamol). Journal of pharmaceutical sciences. 2006 Jan 1;95(1):4-14.DOI: https://doi.org/10.1002/jps.20477

[34] National Center for Biotechnology Information. PubChem Compound Summary for CID 1983, Acetaminophen. https://pubchem.ncbi.nlm.nih.gov/compound/Acetaminophen

[35] National Center for Biotechnology Information. PubChem Compound Summary for CID 5280343, Quercetin. https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin

[36] Liu F, Wang LY, Yu MC, Li YT, Wu ZY, Yan CW. A new cocrystal of isoniazid-quercetin with hepatoprotective effect: The design, structure, and in vitro/in vivo performance evaluation. European Journal of Pharmaceutical Sciences. 2020 Mar 1;144:105216.DOI: https://doi.org/10.1016/j.ejps.2020.105216

[37] George CP, Thorat SH, Shaligram PS, Suresha PR, Gonnade RG. Drug–drug cocrystals of anticancer drugs erlotinib–furosemide and gefitinib–mefenamic acid for alternative multi-drug treatment. CrystEngComm. 2020;22(37):6137-51.DOI: https://doi.org/10.1039/D0CE00353K

[38] Liu F, Wang LY, Li YT, Wu ZY, Yan CW. Protective Effects of Quercetin against Pyrazinamide Induced Hepatotoxicity via a Cocrystallization Strategy of Complementary Advantages. Crystal Growth & Design. 2018 Jun 8;18(7):3729-33.DOI: https://doi.org/10.1021/acs.cgd.8b00576

[39] McGill MR, Williams CD, Xie Y, Ramachandran A, Jaeschke H. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. Toxicology and applied pharmacology. 2012

Nov 1;264(3):387-94.DOI: https://doi.org/10.1016/j.taap.2012.08.015

[40] Kane AE, Mitchell SJ, Mach J, Huizer-Pajkos A, McKenzie C, Jones B, Cogger V, Le Couteur DG, de Cabo R, Hilmer SN. Acetaminophen hepatotoxicity in mice: effect of age, frailty and exposure type. Experimental gerontology. 2016 Jan 1;73:95-106.DOI: https://doi.org/10.1016/j.exger.2015.11.013

[41] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S. Effect of biofield treatment on spectral properties of paracetamol and piroxicam. Chemical Sciences Journal. 2015 Jul 13;6(3).DOI: https://hal.archives-ouvertes.fr/hal-01390464

[42] Latif S, Abbas N, Hussain A, Arshad MS, Bukhari NI, Afzal H, Riffat S, Ahmad Z. Development of paracetamol-caffeine co-crystals to improve compressional, formulation and in vivo performance. Drug development and industrial pharmacy. 2018 Jul 3;44(7):1099-108.DOI: https://doi.org/10.1080/03639045.2018.1435687

[43] Wu N, Zhang Y, Ren J, Zeng A, Liu J. Preparation of quercetin–nicotinamide cocrystals and their evaluation under in vivo and in vitro conditions. RSC Advances. 2020;10(37):21852-9.DOI: https://doi.org/10.1039/D0RA03324C

[44] Hiendrawan S, Veriansyah B, Widjojokusumo E, Soewandhi SN, Wikarsa S, Tjandrawinata RR. Physicochemical and mechanical properties of paracetamolcocrystal with 5-nitroisophthalic acid. International journal of pharmaceutics. 2016 Jan 30;497(1-2):106-13.DOI: https://doi.org/10.1016/j.ijpharm.2015.12.001

[45] Schultheiss N, Newman A. Pharmaceutical cocrystals and their physicochemical properties. Crystal growth and design. 2009 Jun 3;9(6):2950-67.DOI: https://doi.org/10.1021/cg900129f

[46] Mulye SP, Jamadar SA, Karekar PS, Pore YV, Dhawale SC. Improvement in physicochemical properties of ezetimibe using a crystal engineering technique. Powder technology. 2012 May 1;222:131-8.DOI: https://doi.org/10.1016/j.powtec.2012.02.020

[47] Athiyah U, Kusuma PA, Tutik T, Lestari ML, Isadiartuti D, Paramita DP, Setyawan D. Crystal engineering of quercetin by liquid assisted grinding method. JurnalTeknologiUniversitiTeknologi Malaysia. DOI: https://doi.org/10.11113/jt.v81.12639.