SUMMARY & CONCLUSION

- Pure drugs were characterized for particles size, distribution width, surface area, uniformity & zeta potential & found to have higher distribution width, higher mean particle size, very low surface area & broad distribution. These parameters significantly affect the solubility, dissol., dispersion stability, cellular uptake & consistency of performance of the pure drugs.

- HPLC methods for estimation of Quercetin, Rutin, Silibinin, Quercetin-Rutin, Quercetin-Silibinin developed methods were validated for all the analytical variables. β

- A thorough drug-drug/drug-excipient compatibility study was performed & found that there were no significant visual changes, no significant change in the absorption b&s & % remaining was within ±2% of the control samples. Hence, combination of Quercetin-Rutin & Quercetin-Silibinin has not shown any significant physical & chemical instability. Moreover, polymer, poloxamer 188 & poloxamer 407 have also not shown any significant physical & chemical instability with quercetin, rutin & silibinin.

- In the present study, we studied the problem of selecting an optimal method for the preparation of dual flavonoid nanoparticles. Analytic hierarchy process decision-making tool was used to select an optimal method & the results suggested nanoprecipitation method would be an optimal method. The study concludes that the logical ladder process has a pivotal responsibility in selecting an optimal method for the preparation of duo encompassed flavono nanoparticles.

- EPO100 nanoparticles were prepared utilizing nanoprecipitation method by stirring approach. PKN design of experiment has been used to reiterate the critical variables comprising viz-a-viz β polymeric strength organic solvent, organic solvent %, organic phase volume, poloxamer 188 conc., poloxamer 407 conc., aqueous phase qty, beaker amount, routilizing swiftness & stirring extent. The optimized batch was used to prepared plain & Quercetin(Qu NPs), Rutin(Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoparticles.
Prepared plain & Quercetin (Qu NPs), Rutin (Ru NPs), & Silibinin (Si NPs), Quercetin-Rutin (Qu-Ru NPs), Quercetin-Silibinin (Qu-Si NPs) loaded polymeric nanoparticulates were estimated for particle sizing, polydispersity (PDI) & zeta value (mV). Nanoparticles prepared utilizing stirring method were with an average particle size <200 nm, PDI (i.e. uniformity <0.7) & zeta potential >20mV.

Lyophilized powder of prepared Quercetin(Qu NPs), Rutin(Ru NPs), Silibinin (Si NPs), Quercetin-Rutin (Qu-Ru NPs), Quercetin-Silibinin (Qu-Si NPs) loaded polymeric nanoparticles were completely soluble in 50 ml of distilled H2O, whereas equivalent quantity of pure Quercetin was practically insoluble in 50 mL of distilled H2O. Quercetin in prepared polymeric nanoparticles has shown >50 fold increase in aqueous solubility than the free Quercetin. Nanosizing not only reduced the size & so escalated the surface abrath, which in turn have increased the aqueous solubility of Quercetin , Rutin & Silibinin.

Prepared plain, Quercetin (Qu NPs), Rutin (Ru NPs), Silibinin (Si NPs), Quercetin-Rutin (Qu-Ru NPs), Quercetin-Silibinin (Qu-Si NPs) loaded polymeric nanoparticles were spherical in shape. Hence, Quercetin & bio-enhancers like Rutin & Silibinin encapsulated in the polymer matrix will alike in round-morphed & anticipated to enhance the indispensable task of Quercetin & bio-enhancers, release of Quercetin & bio-enhancers from the polymer matrix, transport of Quercetin & bio-enhancers in the body & internalization of Quercetin & bio-enhancers by many folds than the free Quercetin & bio-enhancers.

Stirring approach displayed excellent encapsulation, drug loading & only insignificant amount of Quercetin & bio enhancer were seen as free drug. Hence, prepared Quercetin (Qu NPs), Rutin (Ru NPs), & Silibinin (Si NPs), Quercetin-Rutin (Qu-Ru NPs), Quercetin-Silibinin (Qu-Si NPs) loaded polymeric nanoparticles is expected to display superior pharmacological activities.

Prepared plain & dual drug loaded poly(butyl methacrylate-co-(2-dimethyl aminoethyl) methacrylate-co-methyl methacrylate) nanoparticles were scanned utilizing FT-IR, which confirm the formation of EPO-100 nanonites & Quercetin & bio-enhancers were encapsulated in the polymeric nanoparticles.
All nanoformulations showed >40% release of drugs within 5 mins, >60% release of drugs within 10 mins, >85% release of drugs within 20 mins, >92% release of drugs within 30 mins & >95% release of drugs within 45 mins. Hence, released nanosized quercetin & bio-enhancers are expected to display enhanced aqueous solubility & permeability. Hence, the movement of undissolved quercetin to the intestine will be prevented; thereby hydrolytic degradation of quercetin in the intestine will also be prevented.

Prepared plain polymeric nanoformulation was evaluated for cytotoxicity utilizing MTT assay on BRL 3A rat liver cells (normal liver cells). Prepared plain E-P-O 100 nanoparticles has showed CTC_{50} >1000 microgm/mL. Hence prepared polymeric nanoparticles do not induce any toxicity on normal cells.

**In-vivo** acute oral toxicity study used to brief out the toxicological variables of single dose administration of the prepared nanoformulations. Both the groups of control & handling group not illustrate any transience throughout the observation class. On the whole the monitoring scheme, the animals in any of the groups had not revealed any sort of reaction related abnormal behaviour. Results confirmed that prepared plain Qu NPs, Ru NPs, & Si NPs, Qu-Ru NPs, Qu-Si NPs nanoparticles didn’t precipitate any symptoms of abnormalities at subjected single oral dose & found to be safe.

**In-vivo** sub-acute oral toxicity study was deliberated to gear out the noxiousness of 28 days continuous administration of the prepared nanoformulations. In the sub-acute repeated toxicity experiment module, animal mortalities not observed in any of the study groups throughout the revise epoch. The animals did not show signs of any action related uncharacteristic changes. The resultings depicted thatβ fed of the nanoparticles devoid of obnoxious things in models. No remarkable similarities were pragmatic in body-mass or provisions utilization of the animals of the handling groups When on put side by side with standard groups. Similarly, no significant changes in haematology parameters & blood biochemical arena factor of the animals of the handling groups when compared with that of the control groups. However, on finishing point of the handling animals were killed, necropsy & disease innervating assessment of fundamental appendages such as liver, heart, kidney, brain were performed & result showed that cells were within normal histopathological limits.
ββ In-vitro antioxidant studies were performed to evaluate the scavenging capacity of prepared Quercetin, Rutin, Silibinin loaded polymeric nanoformulation displayed mild % Inhibition in comparison with ascorbic acid. Prepared Quercetin-Rutin & Quercetin-Silibinin dual-loaded polymeric nanoformulation displayed significant % Inhibition of 96.04% & 97.88% respectively in comparison with ascorbic acid. However, Quercetin-Rutin & Quercetin-Silibinin dual-loaded polymeric nanoformulation displayed significantly enhanced % Inhibition in comparison with Quercetin, Rutin, Silibinin loaded polymeric nanoformulation & pure compound. Out of two dual drug loaded nanoparticles, Qu-Si polymeric nanoformulation displayed % inhibition which resembles ascorbic acid’s antioxidant activity. Moreover, the dual-loaded formulation under exploration also exhibit hypo-glycaemic & wound-healing action. Since, Qu-Si polymeric nanoformulation exhibited excellent free radical scavenging activity, its role in cancer medicine may also be explored. This may helps to explain the clinical utility of dual loaded flavono polymeric nanoparticles for the treatment of inflammation & for wound healing.

Prepared Quercetin(Qu NPs), Rutin(Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoparticles were study for its Anti-inflammatory efficacy utilizing complete Freund’s –carrageenan paw edema method. There was no significant differences in paw edema between incept compute (day zero) preceding to adjuvant & just former to the carrageenan injection (day, 6, 0 h). Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) dual loaded polymeric nanoparticlesβ lessened the swelling precipitate in the acute segment, induced 3-6 hr after carrageenan injection (day 6) but pure compound, Quercetin, Rutin & Silibinin loaded polymeric nanoparticles was active only at 7th day. During the chronic phase pure compound had no momentous action in dayz seven, fourteen & twenty eight day. In the chronic phase (days 7-28) only Quercetin-Rutin (Qu-Ru NPs), Quercetin-Silibinin (Qu-Si NPs) dual loaded polymeric nanoparticles was significantly active on all the days.

Prepared Quercetin (Qu NPs), Rutin (Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoparticles were study for its Wound Healing efficacy utilizing excision wound model. The animals treated with the Quercetin-Rutin (Qu-Ru NPs), Quercetin-Silibinin (Qu-Si NPs) dual loaded polymeric
nanoparticles showed faster wound contraction & epithelialization whereas pure compound & Quercetin, Rutin & Silibinin loaded polymeric nanoparticles treated group was showed slower wound contraction & epithelialization.

- Prepared Quercetin (Qu NPs), Rutin (Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoparticles were study for its anti diabetic efficacy utilizing GOD-POD kit method. Diabetic rat treated with Quercetin, Rutin & Silibinin loaded polymeric nanoparticles mild changes in body weight in comparison with diabetic control group. However Diabetic rat treated with Quercetin- Rutin (Qu-Ru NPs)& Quercetin- Silibininβ (Qu-Si NPs) dual loaded polymeric nanoparticles showed tremendous changes in body weight in comparison with diabetic control group. In the efficacy studies significantly decrease in serum sugar stage was attained in diabetic rodent feasted with Quercetin- Rutin (Qu-Ru NPs) & Quercetin- Silibininβ (Qu-Si NPs) dual loaded polymeric nanoparticles as compared toβ diabetic control group. These study results clearly demonstrated that Qu-Ru NPs & Qu-Si NPs dual loaded polymeric nanoparticles bids an effectual per-oral scheme with lessened dose & dozing pace for handling of diabetes & so put forwards the patient compliant.

- Hepatoprotective activities were performed to evaluate the efficacy of prepared Quercetin (Qu NPs), Rutin (Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoparticles. Prepared dual loaded Quercetin-Rutin(Qu-RuNPs),Quercetin-Silibinin(Qu-SiNPs) polymeric nanoformulation displayed enhanced hepato protective activityβ against various toxic agent (Paracetamol,CCL₄ & Et-OH) insitu-intoxicated induced comparison with plain compound & single loaded polymeric nanoformulation. However, out of five prepared nanoformulation, dual loaded polymeric nanoformulation (DLNPs) showed significantly improved hepato protective activity. Moreover Paracetamol intoxicated animals that were treated with single loaded polymeric nanoformulation (SLNPs) & dual loaded polymeric nanoformulation (DLNPs) had improvise histopathological changes as compare to the pure & positive control group.ββ

- Prepared Quercetin (Qu NPs), Rutin (Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoparticles were study for its in-vitro anti-cancer efficacy against human breast adenocarcinoma cells utilizing MTT assay.
Prepared Quercetin (Qu NPs), Rutin (Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoformulations displayed enhanced cytotoxicity against MCF-7 cells in comparison with pure compound. Out of two dual drug loaded nanoparticles, Qu-Si polymeric nanoformulation displayed significant cytotoxicity on MCF-7 cells.

Prepared Quercetin, Rutin, Silibinin, Qu-Ru & Qu-Si loaded polymeric nanoformulations were study for its in-vitro anti-cancer efficacy against multidrug resistant human ovarian cancer (Ovkar3) cells utilizing SRB assay. However, prepared Quercetin, Rutin, Silibinin, Qu-Ru & Qu-Siβ loaded polymeric nanoformulations displayed enhanced cytotoxicity against Ovkar-3 at 80 microgm/mL than the pure compound. Out of five drug loaded nanoparticles, Qu-Ru & Qu-Si loaded polymeric nanoformulation displayed enhanced cytotoxicity against multi-drug resistant Ovkar-3 cells.

Prepared Quercetin, Rutin, Silibinin, Qu-Ru & Qu-Si loaded polymeric nanoformulations were study for its in-vitro antitumor usefulness alongside human cervix-related viz-a-viz malignoma (HeLa) cells utilizing SRB assay. Hence prepared Quercetin, Rutin, Silibinin, Qu-Ru & Qu-Siβ loaded polymeric nanoformulations displayed enhanced cytotoxicity against HeLa at 80 microgm/mL than the pure compound. Out of five drug loaded nanoparticles, Qu-Ru & Qu-Si dual loaded polymeric nanoformulation displayed enhanced cytotoxicity against multi-drug resistant HeLa cells.

Prepared Quercetin, Rutin, Silibinin, Qu-Ru & Qu-Si loaded polymeric loaded polymeric nanoparticles were study for its in-vitro anti-cancer efficacy against human hepatoma (HEPG2) cells utilizing SRB assay. However, prepared Quercetin, Rutin, Silibinin, Qu-Ru & Qu-Siβ loaded polymeric nanoformulations displayed enhanced cell induced abnormalities adjacent to Hepatic-PG2 cells at 80 microgm/mL than the pure compound. Out of five drug loaded nanoparticles, Qu-Si loaded polymeric nanoformulation displayed enhanced cellular level obnoxious reactions against many-drug resistant Hepatic-P,G2 cells.

Prepared Quercetin (Qu NPs), Rutin (Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoparticles displayed enhanced anti-cancer property against mammary cancer in comparison with pure Quercetin in in-vivo study. However, out of three formulations, Qu-Si polymeric nanoformulation showed
significant anti-cancer property. Prepared Quercetin, Rutin & Silibinin, Qu-Ru & Qu-Si loaded polymeric nanoformulations displayed enhanced anticancer property against mammary cancer in comparison with pure compound. However, out of five formulations, Qu-Si polymeric nanoformulation showed significant anticancer property.
LIMITATION OF RESEARCH WORK

- Prepared Single (Quercetin, Rutin & Silibinin) Dual {(Quercetin-Rutin) & (Quercetin-Silibinin)} loaded polymeric nanoparticles are not formulated in to various dosage form (Oral & Intravenous dosage form).

- Phytochemical constituents are not isolated due to its purity hence we procured the isolates from authenticated company.

- Prepared single & dual flavonoid loaded polymeric nanoparticles need to be subjected for lyophilisation process.

- Pharmacokinetic & bioavailability studies of prepared single & dual flavonoid loaded polymeric nanoparticles need to be evaluated.

- A pharmacodynamic study has carried out only on the rodents & to be carried out on human volunteers.

- Chronic toxicity & mutagenicity studies are not established in this research.

- Inflammatory biomarkers need to be done which is lack in the present study due to financial constrained.
**FUTURE SCOPE OF RESEARCH**

- Lyophilisation of prepared nanoparticles for CONVERTING INTO various oral & parenteral formulation.

- Enhancement of Pharmacological activities of prepared Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded polymeric nanoparticles polymeric nanoformulations than the pure Quercetin might be due to:

  1. Natural bio-enhancers (Rutin & Silibinin) might have synergistically enhanced the Pharmacological activities of Quercetin.

  2. Bio-enhancer rutin & silibinin might have suppressed the drug biotransformationatic enzyme CYP P450 3A, liver & small intestinal glucuronidation & sulfation of the quercetin.

  3. Rutin & silibinin along with quercetin might have overturn the multidrug resistance by altering AdenosineTriPhosphate-binding cassette transporter proteins such P-Glycoprotein(P-gp), Multi Drug Resistant Protein like MDRP1, MDRP2 & BCRP & enhanced anti-cancer activity in multidrug resistant human cancer cells such as Övkar3, HeLa & HEPG2.

  4. Nanosizing not only decreases the particles size but also increase its surface area, which in turn increased the aqueous solubility of the drug, which leads to increased its Pharmacological activity of the prepared EPO polymeric nanoformulation.

  5. Prepared Quercetin (Qu NPs), Rutin (Ru NPs), Silibinin(Si NPs), Quercetin-Rutin(Qu-Ru NPs), Quercetin-Silibinin(Qu-Si NPs) loaded poly(butyl methacrylate-co-(2-dimethylamino ethyl) methacrylate-co-methyl methacrylate) (EPO 100 )polymeric nanoparticles released nanosized quercetin & natural bio-enhancers(Rutin & Silibinin) in gastric fluid & intestinal fluid, which in turn increased the aqueous solubility & permeability. Hence, the movement of undissolved quercetin to the intestine might have prevented; thereby hydrolytic degradation of quercetin in the intestine might have prevented.
6. Released nanosized quercetin & natural bio-enhancers might have targeted the affected cells by passive targeting mechanism via enhanced permeability & retention (EPR) effect & thereby enhance its pharmacological activities.

☐ Molecular mechanism need to establish in future research.

☐ Designed nanoparticles necessity to include the chronic toxicity for chronic disease like diabetes & arthritis in future study.

☐ Pharmacokinetic parameter needs to be including in future research.