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Nanosuspension enhances dissolution rate and oral bioavailability of *Terminalia arjuna* bark extract *in vivo* and *in vitro*Fatiqa Zafar¹✉, Nazish Jahan², Khalil-Ur-Rahman³, Muhammad Rafique Asi⁴, Waseeq-Ul-Islam Zafar⁵¹Department of Chemistry, University of Okara, Okara, Pakistan²Department of Chemistry, University of Agriculture, Faisalabad, Pakistan³Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan⁴Food Toxicology Lab, Plant Protection Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan⁵Department of Computer Science, COMSATS University of Information and Technology, Islamabad, Pakistan

ABSTRACT

Objective: To enhance the dissolution rate and oral bioavailability of *Terminalia arjuna* bark extract by formulating its nanosuspension.

Methods: Nanoprecipitation approach was used for the formulation of nanosuspension using polysorbate-80 as a stabilizer. The formulated nanosuspension was assessed for particle size, polydispersity index, zeta potential value and for *in vitro* dissolution study. Oral bioavailability studies were carried out in Wistar male albino rats by administering a single dose (50 mg/kg. b. wt) of the formulated nanosuspension and coarse suspension. The storage stability of the formulated nanosuspension was determined after three months of storage at room temperature and under the refrigerated condition. Mutagenicity assay was carried out to evaluate the toxicity of the formulated nanosuspension using two mutant strains (*Salmonella typhimurium* TA100 and *Salmonella typhimurium* TA98).

Results: The mean particle size of the formulated nanosuspension was 90.53 nm with polydispersity index and zeta potential values of 0.175 and -15.7 mV, respectively. *Terminalia arjuna* nanosuspension showed improved dissolution rate and 1.33-fold higher oral bioavailability than its coarse suspension. The formulated nanosuspension also showed better stability under the refrigerated condition and was non-mutagenic against both strains.

Conclusions: Our study demonstrates that nanosuspension technology can effectively enhance the dissolution rate and oral bioavailability of *Terminalia arjuna* bark extract.

KEYWORDS: *Terminalia arjuna*; Nanosuspension; Bioavailability; Stability; Toxicity; Polysorbate-80

1. Introduction

Recently, nanotechnology has emerged as a marvelous field in the pharmaceutical industry[1] and nanotechnology-based approaches are extensively used to improve the water solubility of poorly soluble drugs[2]. Among various nanotechnological approaches, nanosuspension technology has emerged as an auspicious approach to improve the solubility of drugs and attracted the attention of pharmaceutical researchers[3]. Nanosuspensions are unique, sub-micron colloidal dispersions of nanosized range drug particles stabilized by appropriate surfactants or polymeric stabilizers[4]. The mean particle size of pharmaceutical nanosuspensions varies in the nanorange[1]. Previously, febuxostat nanosuspension showed a particle size of less than 50 nm[5]. Ostwald-Freundlich and Noyes-Whitney equations explained that the particle size reduction to the nanometer range can lead to improved saturation solubility and dissolution velocity. Therefore, the nanoformulation of poorly soluble drugs can improve oral bioavailability compared with traditional formulations[6]. A distinct advantage of nanosuspension is that, along with oral route, they can also be effectively applied through other administrative routes such as pulmonary, ocular

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and parenteral[7] and have shown preeminence over the traditional formulation products in each administrative route[6]. Furthermore, fast-dissolving drug nanosuspensions can substantially improve the stability, safety, therapeutic efficacy and patient compliance[8].

Terminalia arjuna (*T. arjuna*) commonly known as arjun is a traditional medicinal plant of Pakistan, having a variety of biological activities including antioxidant, anti-mutagenic, anti-diabetic and anti-microbial[9] and possessing strong cardioprotective potential[10]. Other pharmacological activities of *T. arjuna* include anti-hyperlipidemic, immune-modulatory, hepatoprotective, analgesic and anti-inflammatory activities[11]. Among various chemical constituents of *T. arjuna*, polyphenols (flavonoids, phenolics, condensed and hydrolysable tannins) are key bioactive compounds accountable for these properties[12].

Quercetin which is a natural flavonoid is present in an adequate amount in *T. arjuna* bark extract[13] with numerous biological and pharmacological properties[14]. Major bioactivities associated with quercetin include antioxidant, cardioprotective, anti-hypertensive, anti-inflammatory, anti-carcinogenic, antiviral and free radicals scavenging potential[15,16]. Although quercetin shows the diverse pharmacological activities, its low water solubility coupled with shorter residence time in the intestine and lower intestinal absorption leads to its poor bioavailability and presents a problem during clinical applications[16,17]. Therefore, the present research formulated the nanosuspension of *T. arjuna* bark extract to enhance the dissolution rate and oral bioavailability of its bioactive phytoconstituents. Pharmacokinetic studies were carried out to evaluate the relative bioavailability of the formulated nanosuspension as compared to coarse suspension. In addition, a mutagenicity assay was performed to check *in vitro* toxicity of the formulated nanosuspension.

2. Materials and methods

2.1. Preparation of *T. arjuna* bark extract

T. arjuna (bark) was collected from University of Agriculture, Faisalabad, identified from plant taxonomist (Dr. Mansoor Hameed) at Department of Botany, University of Agriculture, Faisalabad and a voucher specimen (228-1-2016) was deposited at Herbarium of the Department of Botany, University of Agriculture, Faisalabad. The collected bark of *T. arjuna* was washed with double distilled water, dried under shade and grounded to fine powder. The plant powder was defatted with *n*-hexane in a Soxhlet extractor (Behr Labor-Technik, Germany) for 6-8 h. For the extraction of polyphenolic and flavonoid contents, the defatted plant powder was extracted with ethanol (95%, HPLC grade) by means of Soxhlet extractor. The obtained extract was concentrated using a rotary evaporator (Buchi, CH-9230 Flawil 1, Switzerland) and preserved in refrigerator for future use.

2.2. Formulation of nanosuspension

Nanoprecipitation approach was used for the preparation of nanosuspension[18]. In brief, the plant extract (1 g) was completely dissolved in ethanol (10 mL) as an organic phase and filtered. The resulting organic phase was then injected gradually (1 mL/min) into 100 mL aqueous solution of stabilizer (polysorbate-80, 2% v/v) with constant mechanical stirring (Lab Mechanical Stirrer JJ-1, China) at 6000 rpm for about 6 h at room temperature. For comparative study, coarse suspension of *T. arjuna* was prepared by dissolving plant extract (1 g) in distilled water (100 mL) at room temperature.

2.3. Lyophilization of nanosuspension

The prepared nanosuspension was frozen and lyophilized (FD-1000, EYELA, Japan) for 72 h at -40°C . The freeze-dried nanosuspension was used for the dissolution study.

2.4. Characterization of nanosuspension

The formulated nanosuspension was evaluated for mean particle size (Z-average; nm), polydispersity index (PDI) and zeta potential using dynamic light scattering technique. For this purpose, Nano Zeta sizer (Malvern Instruments, UK) was used.

2.5. Stability testing

To check the storage stability of the formulated nanosuspension, the freshly prepared nanosuspension was poured into two falcon tubes and one of them was kept at room temperature ($25-30^{\circ}\text{C}$), whereas, the other was stored in the refrigerator (4°C) for three months. The physical appearance, mean particle size and PDI values were determined after three months of storage and compared to the results obtained for the freshly prepared nanosuspension.

2.6. Mutagenicity assay

Mutagenicity assay (Ames test) was used for determining the *in vitro* toxicity of the formulated nanosuspension. The test was based on the “Ames bacterial reverse mutation assay” and carried out in liquid culture[19]. For the evaluation of mutagenicity of *T. arjuna* nanosuspension and coarse plant extract, two mutant strains, *Salmonella typhimurium* (*S. typhimurium*) TA100 and *S. typhimurium* TA98 were used. The bacteria were maintained on nutrient agar at ($3 \pm 1^{\circ}\text{C}$) and incubated for about 24 h at 37°C prior to the test.

2.6.1. Reagent mixture preparation

Reagent mixture was prepared by mixing *D*-glucose (4.75 mL), *D*-Biotin (1.19 mL), Davis Mingioli Salt (21.26 mL), *L*-Histidine (0.06 mL) and bromocresol purple (2.38 mL) aseptically in a sterile

bottle. Coarse suspension/nanosuspension, reagent mixture, strains, standard mutagens, and sterile deionized water were thoroughly mixed in bottles at the specific amount (Supplementary Table 1) and inoculated with homogenous culture broth of *S. typhimurium*.

2.6.2. Procedure and result interpretation

The contents of all the bottles were added into separate wells of microtiter plate with 96 wells. Plates were preserved in polythene bags and incubated for 4 d at 37°C. Micro titer plates with blank samples were checked first and remaining plates were checked only when all the wells of blank plate were of purple color indicating that assay was not contaminated. All the remaining plates were then visualized, and all yellow and turbid/partial yellow wells were regarded as positive, whereas, the wells with purple coloration were categorized as negative. Tested sample (nanosuspension/coarse suspension) was regarded as toxic to the tested strains when all the wells showed purple coloration. If the number of positive wells was greater than two times the number of positive wells of the background plate, the sample was believed to be mutagenic.

2.7. In vitro dissolution studies

In vitro dissolution study was performed by the method of Gera et al.[20] with some modifications. For the dissolution experiment, the dissolution apparatus (Pharm Test DT 70, Germany) was used and encapsulated samples (500 mg lyophilized nanosuspension and coarse plant extract) were placed in the dissolution medium (900 mL, 0.1 M phosphate buffer at pH 7.4). The temperature of the dissolution medium was set to (37 ± 0.5)°C and the stirring rate was adjusted to 50 rpm. Aliquots (5 mL) were collected from the dissolution vessel at predetermined time intervals of 15 min up to 2 h. The sink conditions were maintained by adding the same volume of pre-warmed dissolution medium. The amount of drug dissolved (quercetin equivalent) was determined by means of UV-vis spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) at a wavelength of 373 nm (λ_{\max} of quercetin). The concentration of quercetin in the samples was evaluated by using a calibration curve of standard quercetin.

2.8. In vivo bioavailability studies

For bioavailability studies, male Wistar albino rats (250 ± 20) g were used as experimental animals and were kept in the animal house of Clinical Medicine and Surgery Department as per principals of 3R's. Before the experiment, rats were kept for a one-week acclimatization period and fed with normal rat food (grains and rat chaw). All rats were divided into two groups with three rats in each group and were fasted overnight with free access to water. Rats of the first group were administered orally with *T. arjuna* nanosuspension

(50 mg/kg body weight) and rats of the second group with *T. arjuna* coarse suspension at the similar dose. After administration, blood samples (0.5 mL) were collected at specified times (0.5, 1, 2, 4, 6, 12 and 24 h) in sodium heparinized tubes and separated plasma was stored at -20°C for further analysis.

As *T. arjuna* bark extract contains a good quantity of quercetin, quercetin was regarded as an active pharmaceutical ingredient and bioavailability of *T. arjuna* coarse suspension and nanosuspension was determined by using a calibration curve of standard quercetin and results were expressed as "quercetin equivalent". For extraction of quercetin from plasma samples, separated plasma sample (200 µL) was added to an Eppendorf tube containing methanol (400 µL) and hydrochloric acid (25%, 200 µL) and mixed well. The mixture was vortexed for 90 s and incubated at 50°C water bath for about 15 min. The resulting mixture was centrifuged (Bolton scientific, UK) at 10000 rpm for 15 min and the clear supernatant was separated and stored at -20°C for HPLC (Shimadzu, Japan) analysis[21]. All the samples were extracted using the same protocol. The HPLC conditions used for quantification of plasma samples are given in Supplementary Table 2.

Important pharmacokinetic parameters including maximum plasma concentration (C_{\max}) and time to attain maximum concentration (T_{\max}) were evaluated directly from a concentration-time graph. The trapezoidal method was used for calculating the area under the curve (AUC_{0-24h}) using Microsoft Excel 2007.

2.9. Statistical analysis

The dissolution experiments were repeated thrice. For pharmacokinetic studies, there were three rats in each group. The results of the dissolution study, C_{\max} and AUC_{0-24h} were presented as mean ± SD ($n = 3$). Statistical data were analyzed using GraphPad Prism 6 Program (Graph Pad Inc., USA). Mean values were compared using one-way analysis of variance followed by the Tukey *post hoc* test. Differences were considered significant at $P < 0.05$.

2.10. Ethical statement

The rats were treated according to the International Ethical guidelines and the protocol was approved by the Graduate Study Research Board with number GDS/15501-4 dated 09-03-2016.

3. Results

3.1. Formulation of nanosuspension

The formulated nanosuspension was physically stable and clear when freshly prepared. The mean particle size and PDI value

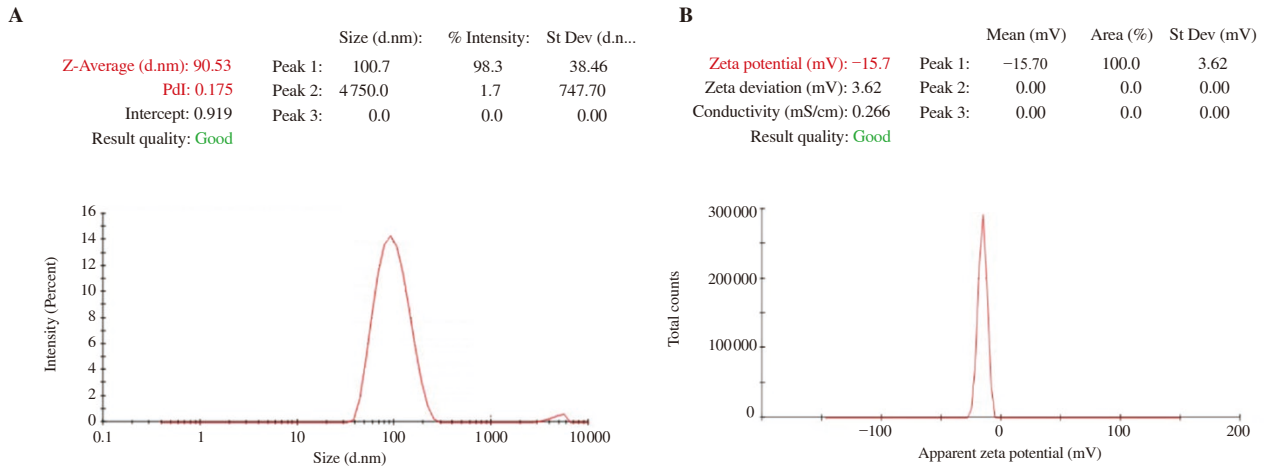


Figure 1. (A) Mean particle size, PDI and (B) zeta potential value of *Terminalia arjuna* nanosuspension. PDI: polydispersity index.

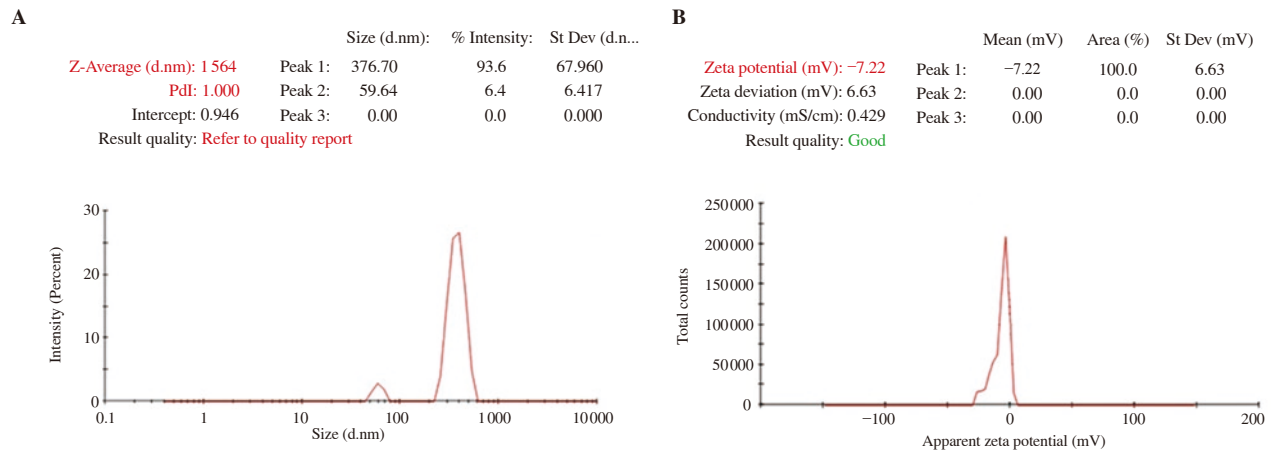


Figure 2. (A) Mean particle size, PDI and (B) zeta potential value of *Terminalia arjuna* coarse suspension. PDI: polydispersity index.

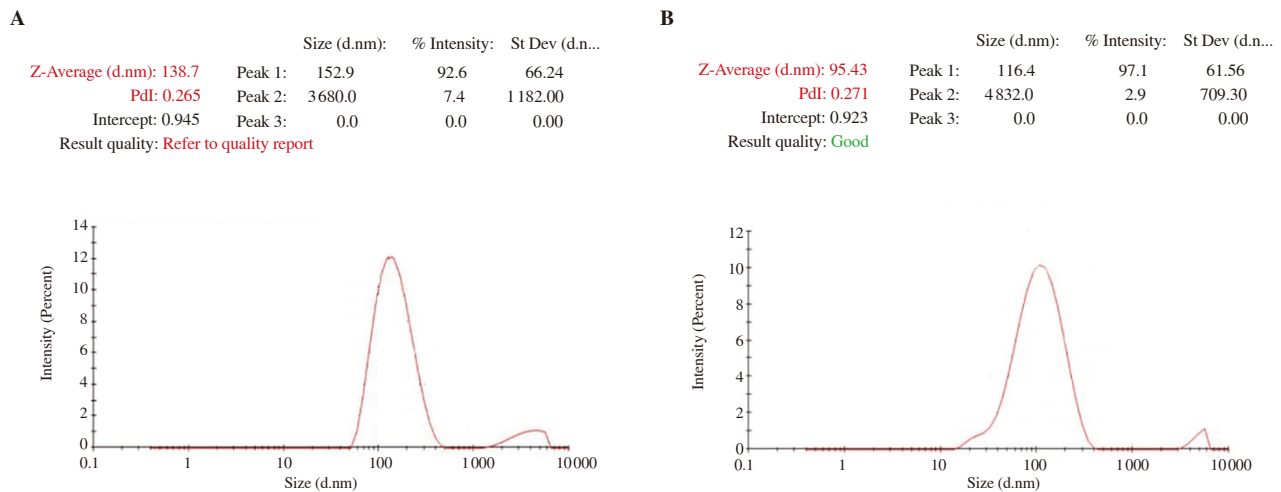


Figure 3. Zeta size and PDI values of *Terminalia arjuna* nanosuspension stored at (A) room temperature and (B) at 4 °C. PDI: polydispersity index.

of the formulated nanosuspension were 90.53 nm and 0.175, respectively (Figure 1A). The zeta potential value of the formulated nanosuspension was -15.7 mV (Figure 1B). The coarse suspension showed the mean particle size of 1 564 nm with PDI and zeta potential value of 1.0 and -7.22 mV, respectively (Figure 2A and 2B). The result indicated that the nanoprecipitation approach could reduce the particle size and PDI values and increase the zeta potential which is responsible for providing long term stability to nanosuspensions.

3.2. Stability studies of *T. arjuna* nanosuspension

No apparent change was noted in the physical appearance of *T. arjuna* nanosuspension when stored at 4°C for three months. However, slight sedimentation was observed in nanosuspension stored at room temperature, which disappeared after slight handshaking. The nanosuspension stored at room temperature showed a significant ($P<0.05$) increase in the particle size (138.7 nm) as compared to nanosuspension stored at 4°C (95.43 nm) (Figure 3A and 3B). A significant ($P<0.05$) increase in the PDI value was also observed for nanosuspension stored at room temperature (0.265) and at 4°C (0.271) (Figure 3A and 3B) as compared to freshly prepared nanosuspension (0.175). These refrigerated conditions were found optimum for better storage stability.

3.3. Mutagenicity assay

Both the tested samples (*T. arjuna* nanosuspension and coarse suspension) were non-mutagenic against both strains (*S. typhimurium* TA98 and TA100). Nanosuspension showed no positive well against TA100 and showed only 4 positive wells against TA98, however, the coarse suspension showed 12 positive wells against TA98 and 14 positive wells against TA100 (Supplementary Table 3).

3.4. In vitro dissolution studies of *T. arjuna* nanosuspension and coarse suspension

Results of the *in vitro* dissolution study of *T. arjuna* nanosuspension and coarse suspension are shown in Figure 4. The curve displayed that the release percentage of bioactive constituent (quercetin) of nanosuspension was higher than that of coarse suspension at each time point. Nanosuspension showed a higher ($P<0.05$) percentage (67.65%) of the drug in the dissolution medium than coarse extract (31.41%) after 1 hour of dissolution experiment. Nanosuspension showed maximum dissolution (84.25%) after 2 h. At that time, the release percentage of the coarse extract was significantly ($P<0.05$) lower (47.67%) than nanosuspension and no more drug was released in the dissolution medium after that.

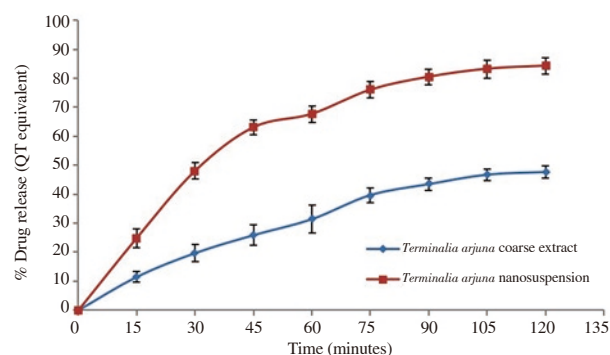


Figure 4. *In vitro* dissolution rate of *Terminalia arjuna* nanosuspension and coarse suspension. Results are given as mean \pm SD ($n = 3$).

3.5. Bioavailability studies of *T. arjuna* nanosuspension and coarse extract

Plasma concentration-time profile after oral administration of *T. arjuna* nanosuspension and coarse suspension is presented in Figure 5 and important pharmacokinetic parameters are given in Table 1. The highest concentration (C_{\max}) of quercetin was observed after 2 hours of dose administration (T_{\max}) in plasma samples of rats treated with nanosuspension (345.89 $\mu\text{g}/\text{mL}$) that was significantly ($P<0.05$) higher than those treated with coarse suspension (268.99 $\mu\text{g}/\text{mL}$). $\text{AUC}_{0-24\text{h}}$ value of the nanosuspension was 1.33-fold higher ($P<0.05$) compared with the coarse suspension.

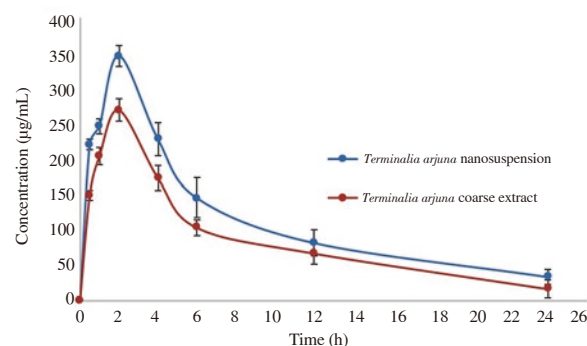


Figure 5. Concentration-time profiles of *Terminalia arjuna* coarse suspension and nanosuspension (quercetin equivalent) after oral administration to experimental rats. Results are given as mean \pm SD ($n = 3$).

Table 1. Pharmacokinetic parameters after oral administration of *Terminalia arjuna* nanosuspension and coarse suspension in rats.

Parameters	Nanosuspension	Coarse suspension
C_{\max} ($\mu\text{g}/\text{mL}$)	345.89 \pm 5.40	268.99 \pm 3.50
T_{\max} (h)	2.00 \pm 0.00	2.00 \pm 0.00
$\text{AUC}_{0-24\text{h}}$ ($\mu\text{g}/\text{mL}$)	2794.51 \pm 17.20	2098.42 \pm 11.50

Values of C_{\max} and $\text{AUC}_{0-24\text{h}}$ are given as mean \pm SD ($n = 3$). T_{\max} is calculated as median. C_{\max} = maximum plasma concentration, T_{\max} = time to achieve maximum plasma concentration, $\text{AUC}_{0-24\text{h}}$ = area under the curve.

4. Discussion

In the present research work, nanosuspension technology was used to enhance the dissolution rate and oral bioavailability of *T. arjuna* bark extract. *T. arjuna* possesses diverse biological activities and is well known for its cardioprotective potential owing to its rich polyphenolic and flavonoid contents[22]. However, the lower aqueous solubility of flavonoid contents of herbal extracts limits their bioavailability in the biological system and hinders their clinical applications[23]. Therefore, in the present study, an attempt was made to enhance the bioavailability of flavonoid contents of *T. arjuna* by particle size engineering. The assumption was based on Noyes–Whitney equation, which states that reduction in particle size improves the bioavailability of drugs in a biological system by effectively increasing the surface area available for dissolution[24].

Nanoprecipitation approach, being universal, simple and reproducible, was employed for the formulation of nanosuspension. The formulated nanosuspension of *T. arjuna* showed required particle size and PDI value, both of which confirmed the formulation of uniform nanosuspension. The good physical stability of nanosuspension was probably due to its smaller particle size (less than 100 nm) and PDI value (less than 0.5)[25], both of which play a key role in governing the physical stability, dispersibility and homogeneity of nanosuspensions. A narrow particle size distribution is essentially required to prevent particle growth due to Ostwald ripening and to retain the stability of nanosuspensions[26,27]. Zeta potential value of a nanosuspension system determines the electrostatic barriers and prevents the nano-sized particles from agglomeration and aggregation[28]. Its value should be at least ± 30 mV for electrostatically stabilized systems or ± 20 mV for sterically stabilized nanosuspension systems[29]. Nanosuspensions with extremely low zeta potential values are at greater risk to become unstable on storage for a long time. In the present study, all three parameters (particle size, PDI and zeta potential value) confirmed the formulation of uniform nanosuspension with required particle size and good physical stability.

Results of stability studies of *T. arjuna* nanosuspension proved that the formulated nanosuspension was stable under both storage conditions (refrigerated and room temperature). Only a slight difference was noted in particle size and PDI values of stored nanosuspension under both conditions that were within the required particle size and PDI values of the pharmaceutical nanosuspension. The good storage stability of *T. arjuna* nanosuspension was prominently due to its smaller particle size and PDI value as discussed earlier. Another reason for better storage stability of the formulated nanosuspension may be the amphiphilic nature of polysorbate 80 (stabilizer) which might enhance the solubility of quercetin contents of *T. arjuna*. As a surfactant, polysorbate 80

provided particle wetting and adsorbed onto the surface, thereby decreasing the interfacial tension between water and quercetin particles. Consequently, water penetrated into the pores of quercetin particles, which may have facilitated the dispersion of particles without solubilization[30]. The result of stability testing illustrated that the prepared nanosuspension did not endure the instability problem. However, nanosuspension stored under refrigerated condition (4 °C) was recommended for better stability. The reason for the increase in particle size and PDI value at room temperature might be the Ostwald ripening, resulting from aggregation or flocculation of the nanoparticles[31]. A higher temperature would increase the kinetic energy or velocity of particle collisions and subsequently increase the energy to overcome the electrostatic repulsion between the nanocrystals which results in aggregation[27]. Present results of stability testing were in agreement with previous studies in which a greater increase in particle size was observed at room temperature as compared to refrigerated conditions[26,27].

The mutagenic activity of coarse plant suspension and nanosuspensions was evaluated by Ames test against two strains of *S. typhimurium*, TA98, and TA100. The mutagenic potential was compared with the background plate and the test substance was considered mutagenic if the number of positive (yellow) wells were two folds higher than the background plate[32]. The mechanism involved in this test is that mutant strains of *S. typhimurium* necessitate histidine for growth. Carcinogenic substances have the ability to change the state in which no histidine is needed for the bacterium to grow. The mutation was measured by using pH as an indicator that changes the color from purple to yellow[19]. In the present study, nanosuspension showed less or no mutagenic activity than coarse suspension. It was interesting to note that the extract of *T. arjuna* which showed non-mutagenic activity in its coarse form become toxic to bacterial strain when formulated into nanosuspension, which revealed the potential of nanosuspension over coarse suspension. To our best knowledge, the mutagenic potential of nanosuspension of herbal extract is studied for the first time. No previous study was found in this regard.

As plants contain a large number of phytoconstituents, it is immensely difficult to determine the concentration of each constituent. Therefore, in the present research work, dissolution and pharmacokinetic studies were carried out using quercetin (which was found to be present in good quantity in *T. arjuna* bark extract) as a reference compound and results were expressed as quercetin equivalent.

The results of the dissolution study depicted better dissolution for *T. arjuna* nanosuspension as compared to its coarse extract. More than 50% of dissolution was achieved in 45 min. The significantly enhanced dissolution rate and improved solubility of *T. arjuna* nanosuspension may be resulted from the increased effective surface

area by excessive particle size reduction. Overall, a 1.77-fold increase was noted in the dissolution rate of *T. arjuna* nanosuspension than its coarse extract. Present results were in accordance with the findings of Ravichandran[33] who studied the dissolution profile of curcumin and inferred that 50% of the curcumin was released into the dissolution medium after 30 min. No previous work was found regarding the dissolution studies of *T. arjuna* nanosuspension.

Results of the pharmacokinetic study illustrated that nanosizing of *T. arjuna* extract leads to substantial improvement in its oral bioavailability. The enhanced bioavailability of *T. arjuna* nanosuspension might be due to direct uptake of nanosuspension through gastrointestinal tract[34]. The possible mechanism involved in such uptake is the diffusion of particles. Drugs having a particle size less than 600 nm allow for efficient uptake in the intestine especially in the lymphoid section of the tissue and consequently bypass the first-pass metabolism in the liver[35]. Moreover, gastrointestinal absorption of drugs which reduces aqueous solubility may be improved by increasing the surface area[36]. A remarkable increase in C_{max} and AUC_{0-24h} also indicated improved *in vivo* exposure of nanosuspension due to extensive reduction in particle size[37], which can be explicated by the greater saturation solubility of nanosized particles because they are absorbed without initial time-consuming step[38]. Present results are in accordance with the previous findings of Hao *et al.*[39] in which resveratrol nanosuspension showed 1.27-fold higher bioavailability than its coarse suspension. To best of our knowledge, nanoformulation and bioavailability studies of *T. arjuna* is carried out for the first time and no previous literature was found in this regard.

In conclusion, nanosuspension technology is a promising approach for the enhancement of oral bioavailability of herbal drugs, and is successfully applied for the formulation of *T. arjuna* nanosuspension. The formulated nanosuspension of *T. arjuna* was found physically stable and non-mutagenic. Moreover, the prepared nanosuspension showed markedly enhanced dissolution rate and illustrated 1.33-fold higher oral bioavailability in rats as compared to its coarse suspension, which proves the efficacy of nanosuspension over coarse suspension.

Conflict of interest statement

We declare that there is no conflict of interest.

Authors' contributions

FZ and NJ designed the study and drafted the manuscript. FZ performed the experiments and wrote the manuscript. NJ and KUR

were responsible for supervision and for critical revision of the article. KUR approved the final manuscript. MRA performed the HPLC analysis regarding *in vivo* experiment. WUIZ analyzed and interpreted data.

References

- [1] Pawar SS, Dahifale BR, Nagargoje SP, Shendge RS. Nanosuspension technologies for delivery of drugs. *Nanosci Nanotech Res* 2017; **4**(2): 59-66.
- [2] Kilor V, Sapkal N, Daud A, Humne S, Gupta T. Development of stable nanosuspension loaded oral films of glimepiride with improved bioavailability. *Int J Appl Pharm* 2017; **9**(2): 28-33.
- [3] He J, Han Y, Xu G, Yin L, Neubi MN, Zhou J, et al. Preparation and evaluation of celecoxib nanosuspensions for bioavailability enhancement. *RSC Adv* 2017; **7**: 13053-13064.
- [4] Wang Y, Zheng Y, Zhang L, Wang Q, Zhang D. Stability of nanosuspensions in drug delivery. *J Control Release* 2013; **172**(3): 1126-1141.
- [5] ElShagea HN, ElKasabgy NA, Fahmy RH, Basalious EB. Freeze-dried self-nanoemulsifying self-nanosuspension (snesns): A new approach for the preparation of a highly drug-loaded dosage form. *AAPS Pharm Sci Tech* 2019; **20**: 1-14.
- [6] Gao L, Zhang D, Chen M, Duan C, Dai W, Jia L, et al. Studies on pharmacokinetics and tissue distribution of oridonin nanosuspensions. *Int J Pharm* 2008; **355**(1-2): 321-327.
- [7] Srivalli KMR, Mishra B. Drug nanocrystals: A way toward scale-up. *Saudi Pharm J* 2016; **24**(4): 386-404.
- [8] Geng T, Banerjee P, Lu Z, Zoghbi A, Li T, Wang B. Comparative study on stabilizing ability of food protein, non-ionic surfactant and anionic surfactant on BCS type II drug carvedilol loaded nanosuspension: Physicochemical and pharmacokinetic investigation. *Eur J Pharm Sci* 2017; **109**: 200-208.
- [9] Jahan N, Rehman KU, Ali S, Bhatti IA. Antioxidant activity of gemmo therapeutically treated indigenous medicinal plants. *Asian J Chem* 2011; **23**: 3461-3470.
- [10] Zafar F, Jahan N, Rahman KU, Khan A, Akram W. Cardioprotective potential of polyphenolic rich green combination in catecholamine induced myocardial necrosis in rabbits. *Evid Based Complement Alternat Med* 2015; **2015**: 734903.
- [11] Ramesh R, Dhanaraj T. GC-MS analysis of bioactive compounds in *Terminalia arjuna* root. *Int J Multidiscip Res Dev* 2015; **2**: 460-462.
- [12] Shanbhag D, Khandagale A. Screening and standardization of *Terminalia arjuna* used as medicine in homeopathy using hptlc method. *Int J Ana Bioana Chem* 2011; **1**: 57-60.
- [13] Pooja S. Production of flavonoids from *Terminalia arjuna* (ROXB.) *in vivo* and *in vitro* tissue cultures. *Int J ChemTech Res* 2014; **6**: 881-885.

- [14]Gao L, Liu G, Wang X, Liu F, Xu Y, Ma J. Preparation of a chemically stable quercetin formulation using nanosuspension technology. *Int J Pharm* 2011; **404**(1-2): 231-237.
- [15]Arshad MS, Sohaib M, Nadeem M, Saeed F, Imran A, Javed A, et al. Status and trends of nutraceuticals from onion and onion by-products: A critical review. *Cogent Food Agric* 2017; **3**: 1-14.
- [16]Penalva R, Gonzalez-Navarro CJ, Gamazo C, Esparza I, Irache JM. Zein nanoparticles for oral delivery of quercetin: Pharmacokinetic studies and preventive anti-inflammatory effects in a mouse model of endotoxemia. *Nanomedicine* 2017; **13**(1): 103-110.
- [17]Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. *Sci World J* 2013; **2013**: 162750.
- [18]Thadkala K, Nanam PK, Rambabu B, Sailu C, Aukunuru J. Preparation and characterization of amorphous ezetimibe nanosuspensions intended for enhancement of oral bioavailability. *Int J Pharm Investig* 2014; **4**(3): 131-137.
- [19]Khan S, Iqbal T, Ahmed N, Jamil A. Antioxidant, hemolytic and mutagenic potential of *Psoralea corylifolia*. *J Animal Plant Sci* 2015; **25**(5): 1451-1456.
- [20]Gera S, Talluri S, Rangaraj N. Formulation and evaluation of naringenin nanosuspensions for bioavailability enhancement. *AAPS Pharm Sci Tech* 2017; **18**(8): 3151-3162.
- [21]Sun W, Mao S, Shi Y, Li LC, Fang L. Nanonization of itraconazole by high pressure homogenization: Stabilizer optimization and effect of particle size on oral absorption. *J Pharm Sci* 2010; **100**(8): 3365-3373.
- [22]Jahan N, Rehman KU, Ali S, Asi MR, Akhtar A. Cardioprotective potential of gemmomodified extract of *Terminalia arjuna* against chemically induced myocardial injury in rabbits. *Pak Vet J* 2012; **32**: 255-259.
- [23]Huang S, Chang WH. Advantages of nanotechnology-based chinese herb drugs on biological activities. *Curr Drug Metab* 2009; **10**(8): 905-913.
- [24]Dizaj SM, Vazifehasl Z, Salatin S, Adibkia K, Javadzadeh Y. Nanosizing of drugs: Effect on dissolution rate. *Res Pharm Sci* 2015; **10**(2): 95-108.
- [25]Abd-Elsalam WH, ElKasabgy NA. Mucoadhesive olaminosomes: A novel prolonged release nanocarrier of agomelatine for the treatment of ocular hypertension. *Int J Pharm* 2019; **560**: 235-245.
- [26]Rachmawati H, Shaal LA, Muller RH, Keck CM. Development of curcumin nanocrystal: Physical aspects. *J Pharm Sci* 2013; **102**(1): 204-214.
- [27]Hong C, Dang Y, Lin G, Yao Y, Li G, Ji G, et al. Effects of stabilizing agents on the development of myricetin nanosuspension and its characterization: An *in vitro* and *in vivo* evaluation. *Int J Pharm* 2014; **477**(1-2): 251-260.
- [28]Karadag A, Ozcelik B, Huang Q. Quercetin nanosuspensions produced by high-pressure homogenization. *J Agric Food Chem* 2014; **62**(8): 1852-1859.
- [29]Papdiwal A, Pande V, Sagar K. Design and characterization of zaltoprofen nanosuspension by precipitation method. *Der Pharma Chemica* 2014; **6**(3): 161-168.
- [30]Sumathi R, Tamizharasi S, Gopinath K, Sivakumar T. Formulation, characterization and *in vitro* release study of silymarin nanosuspension. *Indo Am J Pharm Sci* 2017; **4**: 85-94.
- [31]Thakkar HP, Patel BV, Thakkar SP. Development and characterization of nanosuspensions of olmesartan medoxomil for bioavailability enhancement. *J Pharm Bioall Sci* 2011; **3**(3): 426-434.
- [32]Mohd-Fuat AR, Kofi EA, Allan GG. Mutagenic and cytotoxic properties of three herbal plants from Southeast Asia. *Trop Biomed* 2007; **24**(2): 49-59.
- [33]Ravichandran R. Studies on dissolution behaviour of nanoparticulate curcumin formulation. *Adv Nanoparticles* 2013; **2**(1): 51-59.
- [34]Hussain N, Jaitley V, Florence AT. Recent Advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. *Adv Drug Deliv Rev* 2001; **50**(1-2): 107-142.
- [35]Yuan H, Chen J, Du YZ, Hu FQ, Zeng S, Zhao HL. Studies on oral absorption of stearic acid sln by a novel fluorometric method. *Colloids Surf B Biointerfaces* 2007; **58**(2): 157-164.
- [36]Gursoy RN, Benita S. Self-emulsifying drug delivery systems (sedds) for improved oral delivery of lipophilic drugs. *Biomed Pharmacother* 2004; **58**(3): 173-182.
- [37]Liu D, Pan H, He F, Wang X, Li J, Yang X, et al. Effect of particle size on oral absorption of carvedilol nanosuspensions: *In vitro* and *in vivo* evaluation. *Int J Nanomed* 2015; **10**: 6425-6434.
- [38]Wang Y, Zhang D, Liu Z, Liu G, Duan C, Jia L, et al. *In vitro* and *in vivo* evaluation of silybin nanosuspensions for oral and intravenous delivery. *Nanotechnology* 2010; **21**(15): 1-12.
- [39]Hao J, Gao Y, Zhao J, Zhang J, Li Q, Zhao Z, et al. Preparation and optimization of resveratrol nanosuspensions by antisolvent precipitation using box-behnken design. *AAPS Pharm Sci Tech* 2015; **16**(1): 118-128.