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Enhanced Hepatoprotective Effect of *Adansonia Digitata* Extract on Paracetamol-Induced Hepatotoxicity.

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ABSTRACT

Reconstituted Lyophilized Dry Emulsion Tablets (RLDETs) containing the methanolic extract of the fruit pulp of *Adansonia digitata* L. (Malvaceae) were prepared using edible oils. The prepared tablets were characterized for disintegration time and droplet size after reconstitution. The tablets were then examined for their hepatoprotective activity against paracetamol-induced hepatotoxicity in rats. Treatment of rats with the lyophilized formulations prior to administration of paracetamol caused a significant reduction of the disturbance in liver function. Liver functions were evaluated by ALT, AST, ALP, total bilirubin and total protein measurements. Antioxidant markers and oxidative stress parameters were also evaluated. The histopathological examination of liver tissue of control and treated animals confirmed the enhanced hepatoprotective activity of the prepared RLDETs.

Keywords: *Adansonia digitata,* Hepatoprotective Activity, Liver, Paracetamol, Reconstitued Dry Emulsion Tablets, Lyophilization.

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INTRODUCTION

Adansonia digitata L. (commonly known as baobab) is a tree native to Central Africa and belongs to family Malvaceae. The fruit pulp of *Adansonia digitata* is used in folk medicine as an antipyretic, anti-inflammatory, analgesic and astringent in the treatment of diarrhoea and dysentery [1]. *Adansonia digitata* fruit pulp extract exerts hepatoprotection through amelioration of lipid peroxidation by its scavenging activity of free radicals and enhancement of the antioxidant defense system [2].

Lipid-based drug delivery systems, including emulsions, have recently attracted the attention as potential strategies for enhancing the oral bioavailability of many synthetic and natural drugs by improving their gastrointestinal solubilization. In addition, the lipid components play an important role in enhancing the drugs bioavailability via inhibiting the efflux transporters, increasing the intestinal wall permeability, and enhancing the transport via the lymphatic system with the possible consequence of reducing first pass metabolism [3]. However, liquid emulsions suffer from the problem of thermodynamic instability that could lead to sedimentation or cracking. Thus, the formulation of dry emulsion has emerged as a possible alternative to surmount this problem because the liquid emulsion system is only formed after reconstitution [4, 5].

Dry emulsions can be prepared utilizing different techniques including spray drying [6], rotary evaporation [7] and lyophilization [8]. Previous studies showed that solid supports including gelatin and maltodextrins are effective matrix formers in the formulation of lyophilized tablets [8, 9]. Lyophilization offers the advantages of rapid reconstitution and adequate stability [8].

In this study, reconstituted lyophilized dry emulsion tablets (RLDETs) of the methanolic extract of the fruit pulp of *Adansonia digitata* L. (Malvaceae) were prepared using edible oils, namely; olive and sesame oils aiming to improve the bioavailability of the extract and consequently its hepatoprotective activity.

MATERIALS AND METHODS

Animals

Male adult Wistar rats weighing 200±20g were used for the present work. All animals were fed on standard pellet diet. They were maintained at a 12 h light /12 h dark cycle during the experiment. Rats were kept for acclimatization for seven days before performing the experiment. The study methodologies are in accordance with the Regulations of Research Bioethics on the Living Creatures of the National Committee of Bio. & Med. Ethics, Kingdom of Saudi Arabia.

Drugs and other materials

Paracetamol, Silymarin, Gelatin, Sorbitol, Span 80 and Tween 80 were purchased from Sigma-Aldrich (GmbH, Germany).

Plant material and extract

The fruits were collected from Sudan in March and identified at Faculty of Science, King Abdulaziz University, and a voucher sample was deposited at Natural Products and Alternative Medicine Department, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia, under registration number AD-2014 [2]. The pulp was extracted with methanol (3 × 2000mL) by maceration at room temperature. The combined methanol extracts were concentrated under reduced pressure and kept in refrigerator till use.

Estimation of extract oral dose

From a previous acute toxicity study in rats [2], after oral administration of *Adansonia digitata* extract for seven days, no mortalities were reported up to 2000mg/kg, and hence 1/10th of the maximum dose administered (i.e., 200mg/kg, p.o.) was selected for the present study.

Preparation of reconstituted lyophilized dry emulsion tablets (RLDETs)

RLDETs were prepared by lyophilization of o/w emulsions of the methanolic extract of the fruit pulp of *Adansonia digitata* L. (Malvaceae). 2% (w/v) gelatin solution was used as the aqueous phase of the emulsion. Edible oils, namely; olive and sesame were used as the oily phase. A mixture of Tween 80 and Span 80 in the ratio of 2:1 was used as an emulsifier. Sorbitol was added as a cryoprotectant. Calculated amount of the extract was stirred with the oily phase until a uniform dispersion is obtained. The oily phase is then added to the gelatin aqueous phase containing the surfactant mixture and homogenized for 10 minutes at a speed of 14,000 using an overhead stirrer (Model RW 20 digital, IKA, Germany). The resulting emulsions were poured into PVC tablet blisters to yield unit tablets containing 25 mg extract dose per tablet. The blisters were kept in a freezer at -80ºC for 24 hours, and then freeze-dried for 48 hours using Christ Alpha 1-2 LD Plus Lyophilizer (Martin Christ GmbH, Ostrode am Harz, Germany). The prepared tablets were kept in a dessicator over calcium chloride until further use.

The detailed composition of the prepared formulations (F1 and F2) and the results of the evaluation are presented in table 1.

Table 1: Theoretical Composition and in vitro evaluation of the prepared reconstituted dry emulsion tablets of the methanolic extract of the fruit pulp of *Adansonia digitata*.*

	F1	F2
Gelatin (2%, w/v)	76.60	76.60
Sesame Oil	8.50	-
Olive Oil	-	8.50
Adansonia digitata Extract	10.60	10.60
Tween80/Span 80 (2:1)	4.25	4.25
Average weight after lyophilization (mg)[a]	235 ± 5.88	238 ± 6.32
Globule size after reconstitution (µm) [b]	0.82 ± 0.05	0.96 ± 0.07

* All the listed ingredients are expressed as %, w/w
[a] Results presented as mean ± SD, n=10
[b] Results presented as mean ± SD, n=3

Globule size analysis of reconstituted emulsion

RLDETs were reconstituted using 20 mL distilled water at 37 ± 2ºC. The reconstituted emulsion was evaluated for globule size after standing for 10 minutes using Zetatrac (Microtrac Inc., PA, USA).

Experimental Design

Acute hepatotoxicity was induced by orally administered paracetamol (2g/kg) on the fifth day, 30 min after treatment [10, 11]. Forty eight animals were used and divided into eight groups of six animals each (n = 6) and treated orally as follows:

Group 1 (C): rats were given distilled water orally for seven successive days.
Group 2 (C+B1): rats were given the base of F1 orally for seven successive days.
Group 3 (C+B2): rats were given the base of F1 orally for seven successive days.
Group 4 (P): rats received distilled water orally for seven successive days; on the fifth day animals were given parcetamol orally.
Group 5 (S+P): animals were given oral silymarin (100 mg/kg) for one week [10]; on the fifth day animals were given oral dose of parcetamol.
Group 6 (E+P): rats received extract (200 mg/kg) orally for seven successive days [5]; on the fifth day rats received parcetamol oraly.
Group 7 (F1+P): rats were given F1 (200 mg/kg) orally for seven successive days; on the fifth day animals received parcetamol orally.
Group 8 (F2+P): animals received F2 (200 mg/kg) orally for seven successive days; on the fifth day animals received parcetamol orally.

On the seventh day, two hours after treatments [11], blood samples were collected via retro-orbital plexus. Serum was obtained by centrifugation of blood samples at 4000rpm for 15min then kept at −20°C for biochemical analysis. Finally, the animals were sacrificed by decapitation. The abdomen of the sacrificed rats was cut open and two parts of liver tissues were obtained. The first part was immediately stored at −80°C till it was used for the biochemical measurements, while the second part was embedded in 10% formalin overnight and processed for the histopathologic study.

Evaluation of Liver Function

To evaluate liver function, alanine amino transaminase (ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP) and total bilirubin were measured spectrophotometrically using kit from Roche Diagnostics (Germany). Total protein was measured by a colorimetric method using a kit from Diamond Diagnostics (Cairo, Egypt) following the manufacturer's protocol.

Evaluation of antioxidants

Lipid Peroxide (MDA) was determined spectrophotometrically in liver tissue homogenates [12]. Glutathione (GSH) was determined in the homogenates of liver following the method of Ellman [13]. Superoxide dismutase (SOD) activity was measured according to Marklund [14]. Catalase activity (CAT) was measured in liver tissue homogenate as described by Aebi [15] using CAT assay kits (Bio-Diagnostic, Egypt).

Histopathological Study

Small parts of liver tissues were fixed in 10% buffered formalin. The fixed samples were subjected to routine histological procedures to obtain 5-µm thick paraffin sections. Tissue sections were stained with hematoxylin and eosin stain (H&E) according to standard staining technique then photographed.

Statistical Analysis

The obtained data were represented as mean ± standard deviation (SD). The comparisons between the different groups were performed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test [16]. The difference showing a level of $P < 0.05$ was considered as statistically significant. The data were analyzed using the Statistical Package of Social Sciences (SPSS) program version 16.

RESULTS

Preparation and evaluation of RLDETs

Both formulations yielded successfully dry and elegant tablets that have the adequate strength to be handled. The prepared tablets showed weight uniformity and were rapidly reconstituted into clear emulsion with globule size of less than 1 µm as presented in table 1.

Effect on Liver Functions Measured as ALT, AST, ALP, Bilirubin, and Total Protein

As shown in Table 2, paracetamol produced a significant ($P < 0.05$) increase in ALT, AST, ALP, and total bilirubin levels. Moreover, there was a significant decrease in the total protein when compared with control animals. Silymarin and *Adansonia digitata* extract alleviated these deleterious effects significantly ($P < 0.05$) compared to the paracetamol group. Pretreatment with the RLDETs resulted in a significant ($P < 0.05$) amelioration of hepatotoxicity compared with paracetamol group. This effect was significantly different from the *Adansonia digitata* extract. This means that RLDETs enhanced the hepatoprotective effect of the methanolic extract. There was no significant difference between the two formulations (Table 2).

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Table 2: Effect on serum liver enzymes (ALT, AST, and ALP), total bilirubin, and total protein in paracetamol-induced liver damage in rats.

	ALT (U/L)	AST (U/L)	ALK (U/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
C	59.8 ± 2.1	83.0 ± 4.2	127.5 ± 5.3	0.73 ± 0.07	7.0 ±0.42
C+B1	59.5 ± 1.3	83.3 ± 2.4	126.5 ± 3.4	0.77 ± 0.05	6.9 ± 0.24
C+B2	58.3 ± 1.0	82.3 ± 3.4	128.3 ± 2.2	0.77 ± 0.06	7.0 ± 0.48
P	194.0 ± 4.5*	128.5 ± 6.2*	231.0 ± 3.4*	2.75 ± 0.31*	4.0 ± 0.17*
S+P	62.8 ± 4.0$^{\#}$	88.0 ± 2.2$^{\#}$	150.5 ± 4.4$^{\#}$	1.06 ± 0.19$^{\#}$	6.3 ± 0.29$^{\#}$
E+P	67.0 ± 2.4$^{\#}$	97.3 ± 2.6$^{\#}$	170.3 ± 2.1$^{\#}$	1.85 ± 0.13$^{\#}$	5.5 ± 0.28$^{\#}$
F1+P	63.0 ± 1.8$^{\#\$}$	89.3 ± 5.4$^{\#\$}$	151.0 ± 4.7$^{\#\$}$	1.12 ± 0.13$^{\#\$}$	6.0 ± 0.10$^{\#\$}$
F2+P	63.8 ± 1.0$^{\#\$}$	90.3 ± 2.2$^{\#\$}$	152.0 ± 4.8$^{\#\$}$	1.20 ± 0.08$^{\#\$}$	6.2 ± 0.17$^{\#\$}$

Results were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's post hoc test. $*P < 0.05$ compared to normal control group. $\#P < 0.05$ compared to paracetamol group. $^{\$}P < 0.05$ compared to extract group.

Effect on Liver MDA, GSH, SOD, and CAT

Pracetamol resulted in a significant increase ($P < 0.05$) of MDA levels associated with a significant ($P < 0.05$) decrease in SOD, GSH activities, and CAT levels (Figures 1). These effects induced by paracetamol administration were corrected significantly ($P < 0.05$) upon treating the animals with either silymarin. or *Adansonia digitata* methanolic extract compared to acetaminophen group. Pretreatment with RLDETs showed a significant ($P < 0.05$) improvement compared with *Adansonia digitata* methanolic extract group. There was no significant difference between the two formulations (Figures 1).

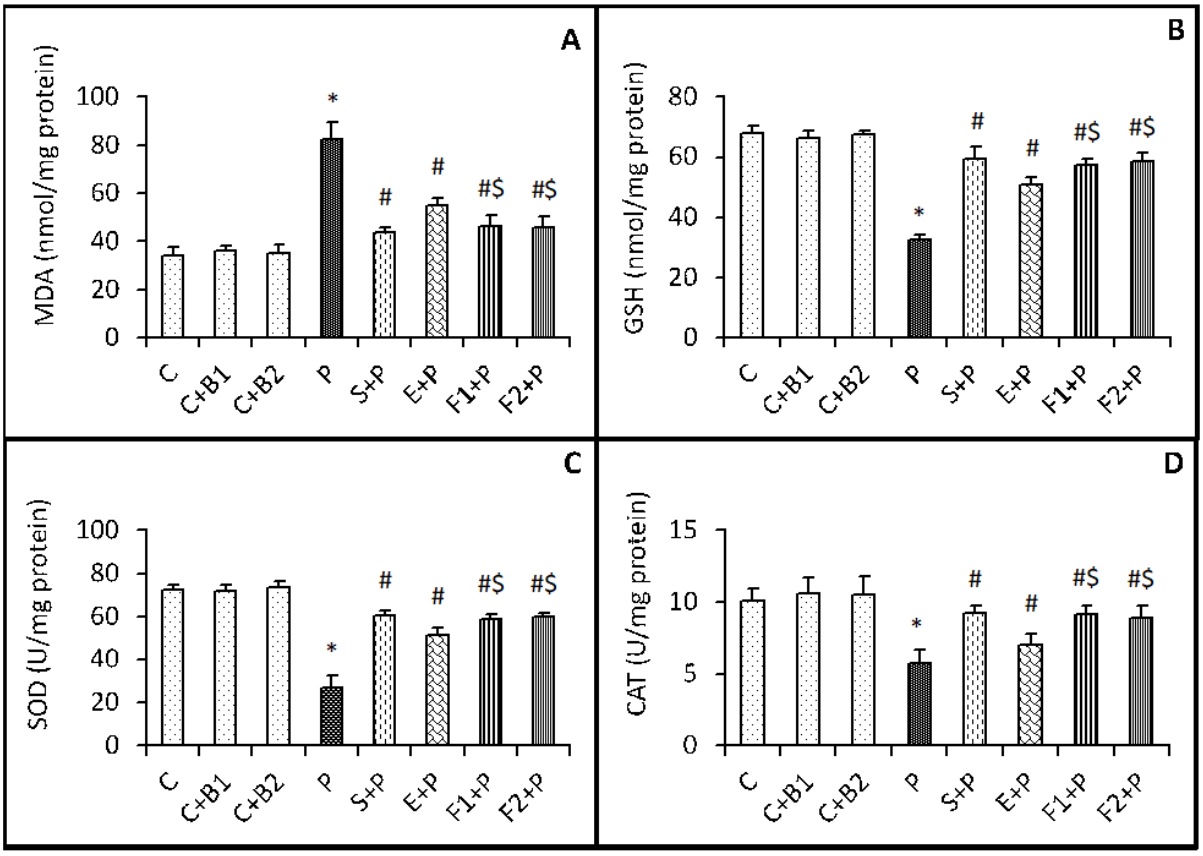

Figure 1: Effect on liver MDA (A), GSH (B), SOD (C) and CAT (D). Each point represents the mean ± SD of six rats. *Significant difference compared with the control group ($P < 0.05$). $^{\#}$Significant difference compared with the paracetamol group ($P < 0.05$). $^{\$}$Significant difference compared with extract group ($P < 0.05$).

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Histopathologic Microscopic Study

In histopathologic study, rat liver tissues of the control groups showed a normal architecture with normal hepatic cells, central vein and sinusoidal dilation (Figure 2(A)). Paracetamol treated animals showed severe necrosis with disappearance of nuclei of hepatic cells (Figure 2(B)). Pretreatment with silymarin showed nearly normal liver structure (Figure 2(C)). Pretreatment with *Adansonia digitata* extract showed parenchyma preservation of hepatocytes with mild necrosis (Figure 2(D)). RLDETs treated groups showed nearly normal hepatic cells (Figure 2(E & F)).

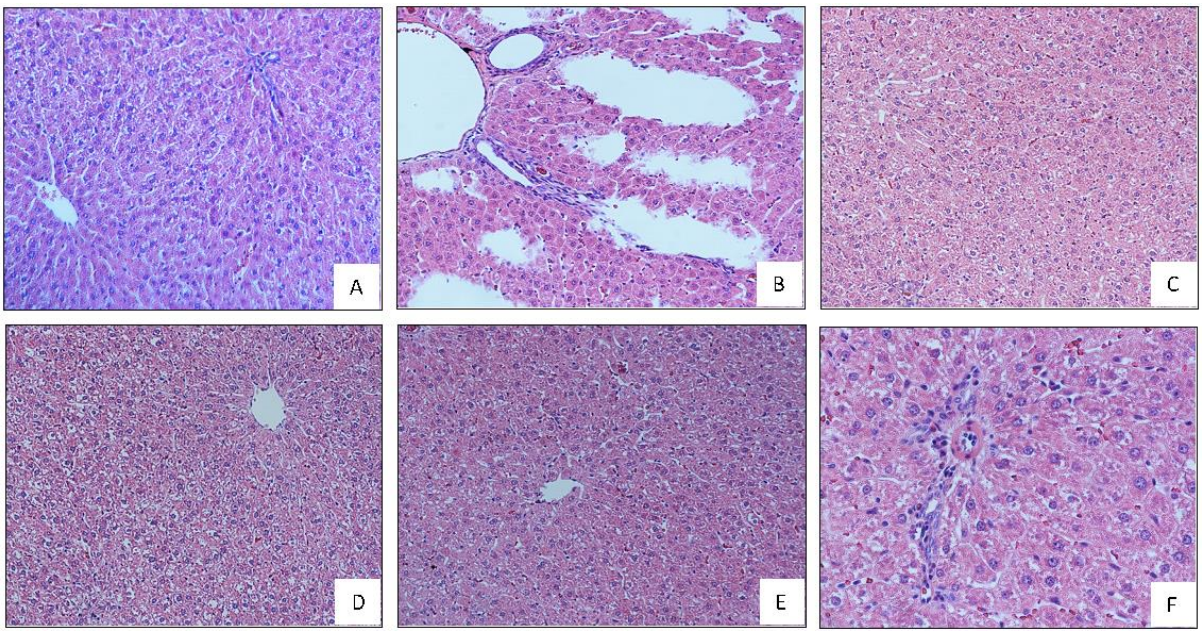

Figure 2: Histopathological study of liver tissue in the different groups of rats. (A) Control group showed normal hepatic tissue (H&E ×200). (B) Paracetamol group showed marked hepatic cell necrosis and wide range of cellular destruction (H&E ×200). (C) Silymarin + paracetamol showed nearly normal liver structure (H&E ×200). (D) *Adansonia digitata* extract + paracetamol showed mild necrosis (H&E ×200). (E) Formulation 1 + paracetamol showed nearly normal liver structure (H&E ×200). (F) Formulation 2 + paracetamol showed nearly normal liver structure (H&E ×400).

DISCUSSION

In this study, the approach of reconstituted lyophilized dry emulsion tablets (RLDETs) was investigated to enhance the hepatoprotective activity of the methanolic extract of the fruit pulp of *Adansonia digitata L.* (Malvaceae). The oils used in the tablets' preparation were chosen based on a preliminary solubility study for the extract in different edible oils (data not shown). Olive and sesame oils exhibited the highest extract solubility. Thus, both oils were used as oily phase for the dissolution of the extract.

One of the most important methods for evaluation of RLDETs is droplet size analysis after reconstitution. The small droplet size of less than 1 µm for both formulations could be attributed to the high emulsification ability of the used surfactant and the application of high speed homogenization during the emulsion preparation [17]. This small particle size indicates increased lipid surface area, thus improving the dispersion of the oily phase and consequently the extract dissolution. The rapid dispersion of the prepared tablets could also be due to the high wetting ability of Tween that allows the oil droplets to present a large surface area, and therefore, to enhance the dissolution rate of the extract. It is worthy to note that the wetting ability is crucial to prevent aggregation of lipid droplets upon contact with the aqueous medium of the gastrointestinal tract [8, 18].

Paracetamol in high dosage is extensively metabolized into N-acetyl-p-benzo-quinoneimine that depletes GSH and leads to hepatocellular death. Moreover, paracetamol induces oxidative stress which is

considered potentially fatal to the cell [19, 20] In the present study, paracetamol hepatotoxicity was confirmed by the high levels of the serum marker enzymes like ALT, AST, ALP, and total serum bilirubin. The increased levels of these enzymes indicates the loss of functional integrity of liver cells membrane [21]. Treatment with silymarin reversed the increased levels of these enzymes. The methanolic extract of the fruit pulp of *Adansonia digitata* showed hepatoprotective effect but less than silymarin. However, RLDETs showed comparable effect to silymarin. Moreover, paracetamol administration produced decrease in the total protein level. This was indicative for liver damage and decreased abilitiy of liver for protein synthesis. However, the serum protein level turned to normal upon pretreatment with silymarin. The methanolic extract of the fruit pulp of *Adansonia digitata* effect was less than silymarin. RLDETs showed comparable activity to silymarin suggesting that these formulations have the ability to protect the liver better than the methanolic extract of the fruit pulp of *Adansonia digitata*.

In the course of paracetamol-induced liver injury, oxidative stress has been suggested. In agreement with previous studies [10], paracetamol administration produced elevation in MDA levels and decrease in antioxidant activity of SOD, CAT, and GSH. The elevated MDA in liver indicates failure of antioxidant defense mechanisms [22]. Cell damage induced by free radicals is prevented by the body defense mechanism which is established by the endogenous antioxidant enzymes, such as GSH, SOD, and CAT [23]. If there is no balance between the production of ROS and antioxidant defenses, oxidative stress results and leads to cell damage. Any compound that has antioxidant properties can prevent or alleviate this damage [20]. In our study, decreased levels of GSH, SOD, and CAT, observed in paracetamol treated rats, are an indication of tissue damage produced by free radicals. Pretreatment with silymarin significantly restored these injurious effects and preserved the normal hepatic physiological mechanisms. The increase in the concentration of these antioxidant enzymes in liver tissues of silymarin-treated rats indicates antioxidant effect of silymarin. RLDETs showed comparable activity to silymarin suggesting that the formulations have a stronger hepatoprotective activity; against free radicals; than the methanolic extract of the fruit pulp of *Adansonia digitata*.

The histopathological findings confirmed the biochemical results. Paracetamol intoxicated rats showed a wide range of hepatic tissue destruction that was manifested by necrosis of hepatocytes and neuclear degeneration. This may be due to free radicals formation and oxidative stress induced by paracetamol. These histopathologic findings were alleviated significantly in the group of rats that were treated with silymarin. RLDETs showed comparable effect to silymarin suggesting that these formulations can protect the liver better than the *Adansonia digitata* methanolic extract.

The enhanced hepatoprotective activity of the RLDETs compared to that of the extract could indicate enhanced bioavailability via the suggested formulations. The small droplet size of the reconstituted emulsion and the high wetting ability of the used surfactant could increase the surface area of the oil droplets. This could result in rapid dispersion, increased dissolution rate and enhanced solubilizing effect of the oil on the extract [8, 24]. In addition, the lipid constituent of the emulsion could interfere with the efflux mechanism, improve the intestinal wall permeability, and enhance the absorption via the lymphatic system [3, 4, 5]. These factors could potentially contribute to the enhanced bioavailability of the extract, and thus, improved hepatoprotective activity.

CONCLUSIONS

RLDETs prepared utilizing edible oils could be a promising safe approach to enhance the bioavailability and the hepatoprotective activity of the methanolic extract of the fruit pulp of *Adansonia digitata*.

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REFERENCES

[1] Caluwé ED, Halamová K, Van Damme P, Afrika Focus 2010; 23: 11-51.
[2] Hanafy A, Aldawsari HM, Badr JM, Ibrahim AK, Abdel-Hady SE, Evidence-Based Complementary and Alternative Medicine 2016; 7 pages.

[3] Kalepu S, Manthina M, Padavala V, Acta Pharmaceutica Sinica B 2013; 6: 361–472.
[4] Gallarate M, Mittone E, Carlotti ME, Trotta M, Piccerelle , Journal of Dispersion Science and Technology 2009; 30: 823–833.
[5] Pongsamart K, Kleinebudde P, Puttipipatkhachorn S, International Journal of Pharmaceutics 2016; 498: 347–354.
[6] Jannin V, Musakhanian J, Marchaud D, Advanced Drug Delivery Reviews 2008; 60: 734–746.
[7] Zu Y, Wu W, Zhao X, Li Y, Zhong C, Zhang Y, International Journal of Pharmaceutics 2014; 477, 148–158,.
[8] Ahmed IS, Aboul-Einien MH, European Journal of Pharmaceutical Sciences 2007, 32: 58–68.
[9] Ahmed I S, Shamma RN, Shoukri RA, Pharmaceutical Development and Technology 2013; 18: 935–943.
[10] Biswas K, Kumar A, Babaria B, Prabhu K, Setty R, Journal of Basic and Clinical Pharmacy 2010 1: 10–15.
[11] Ramachandra Setty S, Quereshi AA, Viswanath Swamy AHM, Fitoterapia 2007; 78: 451–454.
[12] Preuss HG, Jarrell ST, Scheckenbach R, Lieberman S, Anderson RA, Journal of the American College of Nutrition 1998; 17: 116–123.
[13] Ellman GL, Analytical Biochemistry 1970; 46: 237–240.
[14] Marklund SL, The Journal of Biological Chemistry 1992, 267: 6696–6701.
[15] Aebi H, Methods in Enzymology 1984; 105, 121–126.
[16] Katz M, Study Design and Statistical Analysis: A Practical Guide for Clinicians, Cambridge University Press, London, UK, 2006.
[17] Karadag A, Yang X, Ozcelik B, Huang Q, Journal of Agricultural and Food Chemistry 2013; 61: 2130–2139.
[18] Sinswat P, Gao X, Yacaman MJ, Williams RO, Johnson KP, International Journal of Pharmaceutics 2005; 302: 113–124.
[19] Rabiul H, Subhasish M, Sinha S, Roy MG, Sinha D, Gupta S, International Journal of Drug Development and Research 2011; 3: 118–126.
[20] Gini KC, Muraleedhara KG, Asian Journal of Experimental Biological Sciences 2010; 1: 614– 623.
[21] Abraham P, Indian Journal of Biochemistry & Biophysics 2005, 42: 59–62.
[22] Shao HB, Chu LY, Lu ZH, Kang CM, International Journal of Biological Sciences 2008, 4: 8–14.
[23] Prakash J, Gupta SK, Kochupillai V, Singh N, Gupta YK, Joshi S, Phytotherapy Research 2001; 15: 240–244.
[24] Ahmed AIS, Aboul-Einien MH, Mohamed OH, Farid SF, European Journal of Pharmaceutical Sciences 2008; 35: 219-285.