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Formulation of Self Micro-Emulsifying Dry Powder of *Zingiber officinale* (Rhizome) and Its Effect on Hepatoprotective Activity in Mice Model

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Abstract

Liver complication is a major concern in the world. Finding out a new way to combat various liver diseases is very much required. Ginger has been reported to have hepato-protective activity. Here we aimed to formulate an oil-based (self-micro emulsifying) powder to improve the hepatoprotective activity of dry powder of *Zingiber officinale* (Ginger). Four different formulations of self-micro-emulsifying dry powder were prepared by mixing with different excipients. The formulated powder was characterized for the angle of repose, Hausner ratio and compressibility index. The hepato-protective activity of the formulated powder was evaluated *in vivo*. All of the parameters tested for powder characterization showed a good response in terms of flow property and compressibility. Formulated powder exhibited a significant decrease ($p<0.05$) in hepatic enzymes like aspartate transaminase (AST) and alanine transaminase (ALT) in carbon tetrachloride (CCl_4) induced hepatotoxic mice compared to fresh ginger powder group which indicates the enhanced hepatoprotective activity of prepared self-micro emulsifying power in hepatotoxicity.

Key words: *Zingiber officinale* (Rhizome), hepatoprotective, carbon tetrachloride, angle of repose, Hausner ratio, compressibility index.

Introduction

The liver is the second largest organ of our body that helps to regulate various physiological processes including detoxification of xenobiotics (Mondal *et al.*, 2017), regulation of homeostasis, control of growth, nutrient supply, reproduction & maintenance of immunity (Ahsan *et al.*, 2009). The liver complication is the most common cause of death throughout the world. About 29 million people suffer from liver disease in EU (Blachier *et al.*, 2013) and approximately 100 million population in the USA suffer from liver disease (American Liver Foundation 2022). Liver dysfunction is characterized by an elevated level of the liver enzymes like- aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and protein in blood plasma (Woreta and Alqahtani, 2014).

CCl_4 is extensively used for modeling acute toxic liver injury in experimental animals (Bursch *et al.*, 1989). CCl_4 induces oxidative stress (Dahiru *et al.*, 2005), inflammation (Tsai *et al.*, 2009), fatty change (Kadiiska *et al.*, 2000) and fibrosis in the liver through the activation of NF-kB in the liver (Reyes-Gordillo *et al.*, 2007). Longtime exposure leads to coma & even death (Recknagel *et al.*, 1989) and acute exposure to high concentrations of CCl_4 results in degeneration of the liver & kidney (Seifert *et al.*, 1994), while chronic exposure can cause cancer (Rood *et al.*, 2001).

From ancient times, botanical medicines have been used as traditional remedies for the prevention & treatment of liver disease worldwide by herbalists & indigenous healers (Takeoka and Dao, 2003). Among them, ginger was the most common. US FDA listed

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ginger as "Generally Recognized as a Safe" (GRAS) (Langner *et al.*, 1998). *Zingiber officinale* also known as ginger under the family Zingiberaceae is commonly known as a dietary spice which possesses several medicinal properties, such as ailment of indigestion, rheumatoid-osteoarthritis, sore throat, dementia & fever (Liu *et al.*, 2017). It also possesses immuno-modulatory, anti-inflammatory (Grzanna *et al.*, 2005), anti-oxidant, anti-cancer & anti-hyperglycemic properties (Chrubasik *et al.*, 2005; Shukla and Singh, 2007; Ali *et al.*, 2008 and Srinivasan, 2017). The presence of polyphenolic compounds like gingerols, zingerone, shogaols and sesquiterpenoids are responsible for their characteristics of ginger flavor *i.e.*, pungent taste (Baliga *et al.*, 2011).

The previous studies uses ginger oil & extract to ascertain its physiological effect (hepatoprotective cardio-protective, anti-oxidant), so there is limited information about ginger self-micro-emulsifying drug delivery system (SMEDDS) to combat hepatotoxicity in the animal model. Self micro-emulsifying drug delivery system (SMEDDS) refers to the isotropic mixtures of surfactants (Solid/Liquid), hydrophilic solvents, co-solvent & natural or synthetic oils which create fine o/w types emulsions rapidly upon gentle agitation or gastric motility that would be encountered in the digestive tract (Singh *et al.*, 2008; Singh *et al.*, 2009; Boyd *et al.*, 2011). The major advantage of this technique is its ability to skip the initial rate-limiting steps of particle dissolution within the aqueous compartment of GIT (Chouksey *et al.*, 2011). There is less potential for the precipitation of drug particles upon dilution in GIT because of partitioning kinetics which will support the particle to remain in lipid droplets in which the drug is dissolved (Dokania and Joshi, 2015).

Powder flow characterization is a complex process that is affected by several physical properties, and features of processing equipment and needs multiple value or indices to be expressed (Ambadipudi *et al.*, 2019). Flow property characterization is very important during pharmaceutical development (Conceicao *et al.*, 2014). Therefore, the measurement of the angle of repose, Carr's compressibility index or Hausner's ratio (Megarry *et al.*, 2019) has been used to ascertain powder flow properties under various experimental conditions in such a way that resembles large–scale production environment.

The previous studies also show that ginger has an anti-arthritic effect (Funk *et al.*, 2016), gastroprotective effect (Jeena *et al.*, 2016), nephron protective effect (Akinyemi *et al.*, 2018) and protective effect against non-alcoholic fatty liver disease (Lai *et al.*, 2016) in animal model. Therefore, the current study aims to evaluate the hepatoprotective effect of self-micro-emulsifying dry powder of *Zingiber officinale* (Rhizome) induced by CCl_4 in mice models, by estimating some biochemical markers of hepatic injury.

Materials and Methods

Chemicals & materials: Fresh ginger was collected from a local municipal market in Noakhali, Chittagong, Bangladesh. Besides this, Carbontetra-chloride (CCl_4), coconut oil, olive oil, tween-80, poly ethylene glycol (PEG-400), aerosil & colorimetric kit for the biochemical assay were collected from Sigma-Aldrich chemical company.

Preparation of self-micro-emulsifying dry powder of ginger & its flow property characterization: After the collection of fresh ginger from a local market, it was chopped, dried, crushed & sieved through 100 sieve mesh to get uniform dry powder. Then 4 (four) different formulations of SMEDDS were developed using different ratios of excipient (coconut oil, tween-80, PEG-400 & aerosil) and garlic powder (Table 1). Finally flow properties of both garlic powder and different formulations of SMEDDS were characterized through the measurement of angle of repose, Hausner's ratio and Carr's compressibility index.

Animal and experimental design: 35 adult male Swiss albino mice weighing about 25-30 gm were collected from International Centre for Diarrheal Disease Research, Dhaka, Bangladesh. All mice were housed in rectangular polypropylene cages (50 × 35 ×

20 cm) and acclaimed under prescribed laboratory conditions of 23-25 °C, 50-55% RH & light illumination of 12/12 h dark/light cycle with proper access of food and water during the experiment period. The experiment was carried out following ethics and guidelines approved by the ethics committee of the university (Ethical Clearance Reference Number: NSTU/SCI/EC/2022/108).

Table 1. Amount of ginger powder (mg) and each excipient (mg) used (Unit formulation).

Formulations	Raw ginger (Crude drug)	Coconut oil (emulsifier)	Tween 80 (surfactant)	PEG 400 (co-surfactant)	Aerosil (glidant)	Total
Formulation-1 (F-1)	160	5	10	15	10	200
Formulation-2 (F-2)	160	8	10	12	10	200
Formulation-3 (F-3)	160	12	10	8	10	200
Formulation-4 (F-4)	160	15	10	5	10	200
Fresh Ginger (FG)	200	0	0	0	0	200

After completion of one week's acclimatization, the mice were randomly divided into 7 (seven) groups each of 5 (five) mice as follow :

Group I (Normal Control group): The animals receiving no treatment.

Group II (Toxicant control group): The animals given 0.2 % CCl_4 using the dose of 8 ml/kg BW.

Group III: The animals given orally raw ginger powder alone using the dose of 300 mg/kg BW.

Group IV-VII: The animals received orally 4 different SMEDDS formulations of ginger powder (different excipient ratios) using the dose of 300 mg/kg BW.

On the 7^{th} day, after two hours of the last treatment with the respective test sample, the animal was anesthetized using chloroform and finally dissected. The blood sample was collected through cardiac puncture followed by serum separation. Different organs such as the heart, kidney, liver and spleen were collected, weighed and preserved in formalin for future analysis.

Biochemical assay: After collection, blood samples were allowed to stand at room temperature for 30 minutes and were allowed to clot. It was then centrifuged at 3500 rpm for 10 minutes in a centrifuge machine and serum was collected for the determination aspartate transaminase (AST) and alanine transaminase (ALT). Liver biomarkers were estimated using laboratory diagnostics kits (Germany) in a semi-automatic chemistry analyzer (Mindray BA-88A) following the procedure given in the respective manufacturing protocol.

Data analysis: Relevant statistical analysis was performed by Student's t-test using GraphPad Prism software version 9.00 for Windows (GraphPad Software, La Jolla, CA, USA). Results are expressed as means ± standard error of the mean (SEM).

Results and Discussion

As it was inconvenient to measure a such small amount of excipient and to prepare a 200 mg formulation, we prepared 50 times each of the 200 mg formulations. (200 mg * 50 = 10 g). The amount of each excipient used to prepare 10 g of each formulation is shown (Table 2).

Fixed funnel method was employed to determine the angle of repose. On the other hand, both bulk density (BD) and tapped density (TD) were used for Hausner ratio (HR) and compressibility index (CI) determination. Formulation shows passable to good flow properties in the case of angle of repose & shows fair to excellent flow properties in the case of Hausner ratio and compressibility index (%) (Table 3).

To promote hepatotoxicity in experimental animals, the liver was damaged using a single dose of 0.2% carbon tetrachloride (8ml/kg.BW) peritoneally with olive oil on the 5^{th} day to each group except the control group; the control group was given only olive

oil (8ml/kg.BW) on 5th day peritoneally. Administration of CCl_4 significantly rises plasma AST and ALT levels.

After treatment with a different formulation of ginger, there have significant fall in plasma AST levels (Table 4) for the different formulations, but no significant difference was observed in fresh ginger (FG) as compared to the toxicant control group. Similarly, the plasma ALT level (Table 4) has no significant change in the case of FG, while the value falls sequentially in different formulations.

Table 2. Amount of ginger powder (g) and each excipient (g) used (Batch formulation)

Formulations	Raw ginger (crude drug)	Coconut oil (emulsifier)	Tween 80 (surfactant)	PEG 400 (co-surfactant)	Aerosil (glidant)	Total
F-1	8	0.25	0.5	0.75	0.5	10
F-2	8	0.4	0.5	0.6	0.5	10
F-3	8	0.6	0.5	0.4	0.5	10
F-4	8	0.75	0.5	0.25	0.5	10
FG	10	0	0	0	0	10

Table 3. Angle of repose, Hausner ratio (HR), compressibility index (%) (CI) of different formulations & its relative flow property.

Formulations	Angle of repose (Degree)	Flow property	HR	CI (%)	Flow property
F-1	38.7	Fair	1.04	4.08	Excellent
F-2	36.38	Fair	1.19	16.37	Fair
F-3	34.4	Good	1.02	2.08	Excellent
F-4	43.15	Passable	1.08	8.16	Excellent
FG	41	Passable	1.18	15	Good

Table 4. Plasma AST and ALT levels of different formulations.

Group	AST			ALT		
	Average (unit/liter)	SEM	P Value	Average (Unit/Liter)	SEM	P Value
ND	182.5	6.207		58.06	3.731	
TC	276.6	7.438	0.0001	79.63	4.173	0.0063
F-1	212.9	7.703	0.0006	76.68	3.495	0.6023
F-2	230.1	9.383	0.0082	70.13	3.669	0.1382
F-3	185.2	9.430	0.0003	59.33	2.838	0.0069
F-4	197.3	5.313	0.0001	58.32	1.970	0.0016
FG	258.6	14.76	0.3504	79.28	4.157	0.9555

Values of AST of different formulation (FC) group were significantly differed from toxicant control (TC) group, where formulation group F-2 ($p=0.0082$) were significantly ($p<0.05$) varied from toxicant control group (TC). On the other hand, formulation group F-1 ($p=0.0006$), F-3 ($p=0.003$) & F- 4 ($p=0.0001$) were moderately significant ($P<0.005$). There's no significant difference observed

in case of fresh ginger (FG). Similarly, values of ALT of formulation group F-3 (p=0.0069) & F-4 (p=0.0016) were significantly (p<0.05) different from toxicant control group (TC). But there's no significant difference observed in case of formulation group F-1 (p=0.6023), F-2 (p=0.1382) & FG (p= 9555) group. The level of significance of both AST & ALT levels also represented in Figures 1A and 1B, respectively.

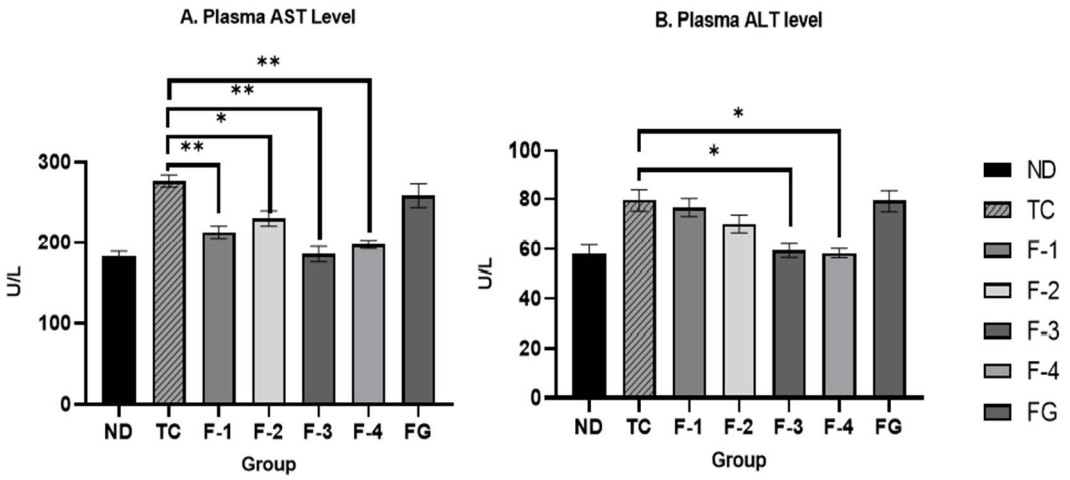

Figure 1. Serum biochemical profile A. Plasma AST level (U/L) and B. Plasma ALT level (U/L) for different formulation of ginger.

Liver, the largest visceral organ & gland of the human body (Heidarian *et al*., 2014), plays a vital role in several physiological processes like- metabolism, secretion and storage & possess the excellent capacity of detoxifying toxic chemicals into non-toxic even useful ones (Abdel-Azeem *et al.*, 2013). Hepatotoxic mechanism of the liver is characterized by its ability to metabolize & generate reactive toxic metabolites through the hepatic microsomal enzymatic system (CYP family) (Gonzalez, 1992). Xenobiotics are usually converted into inert metabolites after microsomal metabolism which is later excreted from the body, but some of them become more reactive toxic compounds than the parent compounds (Abdel-Azeem *et al.*, 2013). Liver damage is characterized by the elevated level of different hepatic biochemical markers like- SGPT, SGOT, ALP, AST, CRP and total bilirubin level which leak into the bloodstream when liver cell undergo damage (Kasdallah-Grissa *et al*., 2007).

Carbon tetrachloride is a well-known xenobiotic responsible to induce hepatotoxicity in various experimental animals (Mansour, 2000). It causes noticeable upregulation of serum biochemical markers of liver damage like- ALT, AST, and ALK (Anand *et al.*, 1992) (Sturgill and Lambert, 1997). Metabolism of CCl_4 is performed by hepatic cytochrome P450 enzymes to yield highly reactive trichloromethyl peroxyl (CCl_3O_2) and trichloromethyl (CCl3) radical leading hepatotoxicity. Both radicals are responsible for liver cell necrosis through lipid peroxidation which in turn leads to excessive deposition of collagen in the liver, ultimately liver fibrosis through binding with cellular macro-molecules like- nucleic acid, carbohydrates, proteins, and lipids (Weber *et al*., 2003; Basu, 2003).

Ginger has been shown to exert a hepatoprotective effect against carbon tetrachloride- acetaminophen-induced hepatic injury (Yemitan and Izegbu, 2006) and reduce serum ALP, ALT, and AST levels supporting its antioxidant as well as a membrane stabilizing properties (Bhandari *et al*., 2003). Water and ethanolic extract of ginger show antioxidant properties as it contains polyphenolic compound zingiberene and oleoresin (Stoilova *et al.*, 2007). Besides this presence of vitamin C and flavonoids

also contribute to its antioxidant activity (Oboh *et al*., 2012). Moreover, active ingredients of ginger like gingerols and gingerol analogs (shogaols and paradols) reduce anti-inflammatory response through inhibition of prostaglandin and leukotriene synthesis (Nurtjahja-Tjendraputra *et al*., 2003). Gingerol also regulates the biosynthesis of glutathione by controlling the expression of γ -glutamyl-cysteine ligase, an antioxidant enzyme (Lee *et al*., 2011).

There are also have researches on the hepato-protectivity of methanolic extract of ginger in mice model, but self-micro-emulsifying drug delivery system (SMEDDS) is more effective due to its improved bioavailability pattern with an increment of solubility, absorption, reduction of degradation and gastric irritation (Wei *et al.*, 2010). For this, the main focus of our study is to develop the self-micro-emulsifying drug delivery system (SMEDDS) of ginger powder and its flow property characterization through measurement of angle of repose, Hausner ratio & compressibility index, so that more effect against hepatic injury could be found.

In this study, the values of angle of repose, Hausner ratio, and compressibility index (Table 3) for each of the formulations were at a satisfactory level which means that the formulated self-micro-emulsified powder possessed good flow properties. The flow property of a drug carries significant importance from the pharmacological perspective of the dosage form. It is very important to get the satisfactory pharmacodynamic and pharmacokinetic activity of a drug.

In the hepatoprotective study, we measured the concentrations of two different liver enzymes-aspartate transaminase (AST) and alanine transaminase (ALT) level in mice models. Here we noticed a very pronounced difference in concentrations of these two enzymes between the toxicant control group and treatment groups of different formulations. Each of the formulations significantly reduced both AST & ALT (Table 4) enzyme levels compared to the toxicant control group except the formulation of fresh ginger. There were no excipients used in that group. So, all of the different formulations of different excipient ratios showed hepatoprotective activity.

Conclusion

Self-micro-emulsifying powder of *Zingiber officinale* (Rhizome) showed a remarkable hepato-protectivity through lowering the hepatic bio-marker *i.e.*, AST, ALT level. The alarming rate of increased liver diseases has become a serious health concern over the world in recent years. Therefore, self-micro-emulsifying powder of *Zingiber officinale* (Rhizome) could be a potential drug for liver complications.

Conflict of interest

The authors declare no conflict of interest.

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