

# Evaluation of Antioxidant Potential of Zingerone against High Fructose Diet Induced Non-Alcoholic Steatohepatitis in Rat Model

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**Abstract**—Zingerone was proven to possess many pharmacological functions including hepatoprotective, antiparasitic, antiflarial, antimicrobial, antidiabetic and radio protective properties. The aim for this study was to evaluate the antioxidant potential of zingerone against high fructose diet induced NASH. A total of 24 rats were used in this *in vivo* study. The rats were divided into four groups ( $n=6$ ) and treated as follows for 8 weeks: Group 1 (CON): Rats received normal standard pellet diet with water, Group 2 (ZIN): Rats received normal pellet diet and zingerone (100 mg/kg body wt /day), Group 3 (HFD): Rats received high fructose diet (40% of fructose and rodent chow with drinking water (25% fructose). Group 4 (HFD+ZIN): Rats received high fructose diet and zingerone (100 mg/kg body wt /day). DPPH radical scavenging activity and total phenolic content assay were done to determine antioxidant potential of zingerone as for the preventive effect of NASH. The results indicated that the zingerone (100 mg/kg body weight) treated group showed the highest scavenging activity in both normal diet rats (ZIN): and high fructose fed rats (HFD+ZIN) which was found to be 129%, and 125% respectively. The total phenolic content and gallic acid concentration was found to be higher in the zingerone treated group (ZIN) indicating the antioxidant potential of zingerone. Hence zingerone may have antioxidant potential against high fructose diet induced non-alcoholic steatohepatitis in rats.

**Keywords**— Non-alcoholic Steatohepatitis (NSAH), high fructose diet, zingerone, total phenolic content, DPPH.

## I. INTRODUCTION

Ginger is one of the most common used condiments and a natural drug which is a traditional medicine, containing some active constituents used for the purpose of treating numerous diseases<sup>1</sup>. Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) is one of the active components in the ginger which is nontoxic compound with varied pharmacological activities. Studies have reported that the influence of ginger rhizome (*Zingiber officinale*) on survival, glutathione and lipid peroxidation in mice after whole-body exposure to gamma radiation, the antioxidant action of ginger has the major possible mechanism for the protective actions of the plant against toxicity and lethality of radiation<sup>2</sup>.

Non-alcoholic steatohepatitis (NASH) is a common, often “silent” liver disease. It occurs in people who drink little or no alcohol. The major feature in NASH is fat in the liver, along with inflammation and damage. Nevertheless, NASH can be severe and can lead to cirrhosis, in which the liver is permanently damaged and scarred and no longer able to work properly. NASH is considered to be the liver expression of metabolic syndrome diseases related to diabetes mellitus type 2, insulin resistance, central (truncal) obesity, hyperlipidemia (low high density lipoprotein cholesterol, hypertriglyceridemia) and hypertension. The prevalence of NAFLD is also reported in the previous research which stated that 6.3% to 33% of the general population is having the disease worldwide and prevalence in Asian countries ranges from 6% to 25% including Malaysia, India and Singapore<sup>3</sup>.

NASH is associated with metabolic disorder, so it is difficult to cure it with single drug therapy.

The use of traditional medicine is shown to have treated various liver diseases. Medicinal herbs are known for their multifaceted implications and thus can form an effective treatment schedule against NASH (2). Plants extract or those herbal extract that have antioxidant, antidiabetic and antihyperlipidemic properties shown to ameliorate symptoms of NASH. According to the past study, oxidative stress is the vital mechanism in non-alcoholic steatohepatitis (NASH). Development of NASH associated with the relationship between oxidative stress and antioxidant enzymes, which they measured the level of the oxidative stress and antioxidant status: glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and superoxide dismutase (SOD). So it will be interesting to investigate the antioxidant potential of Zingerone against high fructose diet induced nonalcoholic steatohepatitis in rat model<sup>4</sup>.

## II. MATERIALS AND METHODS

### Chemicals

Zingerone was obtained from Sigma Chemical Co., St Louis, MO, USA. All the assay kits were procured from Qualichem (Sea) Sdn. Bhd., Malaysia. All other chemicals and reagents used were of analytical grade.

### Animals

An *in vivo* experiment was conducted with 24 male Wistar rats weighing 150-180 g approximately. The animals were housed in propylene cages with standard conditions at the Central Animal House. The rats were acclimatized for 1 week

and were provided with the standard pellet diet and water before the dietary intervention. Rats were randomly assigned into 4 different groups (n=6) and treated as follows for 8 weeks: Group 1 (CON): Rats received normal standard pellet diet with water, Group 2 (ZIN): Rats received normal pellet diet and zingerone (100 mg/kg body wt /day), Group 3 (HFD): Rats received high fructose diet (40% of fructose and rodent chow with drinking water (25% fructose). Group 4 (HFD+ZIN): Rats received high fructose diet and zingerone (100 mg/kg body wt /day). After the treatment the rats were euthanized, blood was obtained and plasma was separated and diluted with phosphate buffer (pH:6.0) and utilized for further assays.

*Total phenolic content (TPC) assay (Folin-Ciocalteu Method)*

The Folin–Ciocalteu (F–C) reagent is sensitive to reducing compounds, polyphenols and thus produces a blue color complex. Total phenolic content was expressed as mg Gallic acid Equivalents (GAE). Rat plasma was diluted (1:50) with phosphate buffer pH 6.0 and used for the TPC assay.

*Gallic acid standard curve*

Gallic acid standard solution was prepared by dissolving 0.1 g of Gallic acid in 10 ml of methanol. A serial dilution with methanol was performed to have the concentration of 0.625, 1.25, 2.5, 5 and 10 mg/ ml. The absorbance was taken at the wavelength of 750 nm using spectrophotometer. Distilled water was used as blank. The concentrations of gallic acid in the samples were determined by using an equation that was obtained from the standard gallic acid curve ( $y = mx + c$ ). A gallic acid standard curve was developed.

*Total Phenolic Assay*

For the TPC assay, to about 200 µL of the rat plasma, 1.5 ml of 10% of Folin-Ciocalteu solution of was added and the test tubes were kept in the dark place for about 5 minutes then 1.5 ml of 5% Na<sub>2</sub>CO<sub>3</sub> was added to it and mixed well. The test tubes were incubated for 2 hours in dark, the absorbance was measured by using spectrophotometer at a wavelength of 750 nm. Distilled water served as a blank.

*DPPH Radical Scavenging Activity*

DPPH assay measures the radical scavenging activity of antioxidant compounds following the reduction property of the compounds. Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is: based on the theory that antioxidant a hydrogen donor, ZH is the reduced form and A• is free radical produced in the first step:  $Z^{\bullet} + AH = ZH + A^{\bullet}$ <sup>45</sup>. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. It is used to quantify antioxidants in complex biological systems, for solid or liquid samples. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Eugenio et al (2012)<sup>6</sup>. 150 µL of DPPH solution 150 µL was added to the sample and stored in the dark for 20 minutes. Later the absorbance was measured at 516 nm in a spectrophotometer. Methanol served as blank.

The percentage of scavenging activity (AA %) was determined based on the equation:

$$AA\% = 100 - [ (Abs_{sample} - Abs_{blank}) \times 100 / Abs_{control} ]^7$$

*Statistical analysis*

Data was analyzed using SPSS software package, SPSS IBM version 23 for windows. Results were statistically analyzed based on three replicates done for each of the three biochemical studies. The data were expressed as mean ± SE. Comparisons of the determined variables among all the grouped data were conducted by one-way analysis of variance (ANOVA) at (p< 0.001) for multiple comparisons.

III. RESULTS

*Gallic acid concentration*

As indicated in table 1 Group 2 showed the highest concentration of Gallic acid (4.44 mg GAE). Group 3 rats receiving only fructose diet was found to have the lowest concentration of gallic acid in the sample 1.32 mg GAE. The highest gallic acid content were found to be in the gingerone treated group when compared to the control group.

TABLE 1. Concentration of gallic acid (mg GAE).

Group	Concentration of Gallic acid (mg GAE)
1 (CON)	1.89
2 (ZIN)	4.44
3 (HFD)	1.32
4 (HFD + ZIN)	1.89

Values of gallic acid equivalents (mg GAE) in control and treated groups. (1.CON: Control;2. ZIN: Zingerone (100 mg/kg body weight), 3. HFD: (40% of fructose and rodent chow with drinking water (25% fructose), 4. (HFD + ZIN): High fructose diet and zingerone at the dose of 100 mg/kg body weight)

*Total Phenol Content*

Table 2 represents the total phenol content in the control and treated groups. The highest phenolic content were found to be in the gingerone treated groups which was recorded as 0.61±0.001 and 0.61±0.160 respectively. The values were found to be 0.48±0.002 and 0.49±0.003 in the control and high fructose diet groups which was significantly lower (p< 0.001) when compared to the gingerone treated groups.

TABLE 2. Total Phenol content (Mean±SD).

Group	Total Phenol Content (Mean±SD)
1 (CON)	0.48±0.002*
2 (ZIN)	0.61±0.001
3 (HFD)	0.49±0.003*
4 (HFD + ZIN)	0.61±0.160

Mean ±SD values of total phenolic content in control and treated groups. (1.CON: Control;2. ZIN: Zingerone (100 mg/kg body weight), 3. HFD: (40% of fructose and rodent chow with drinking water (25% fructose), 4. (HFD + ZIN): High fructose diet and zingerone at the dose of 100 mg/kg body weight)\* (p< 0.001).

*DPPH Radical Scavenging Activity*

Table 3 indicates the DPPH radical scavenging activity of the control and treated groups. Group 2 (ZIN) showed the highest radical scavenging activity compared to the other groups. For the gingerone treated high fructose diet group (HFD + ZIN), the radical scavenging activity was found to be 125%, there was significant (p<0.001) difference when

compared to the control (CON) and high fructose treated group (HFD).

TABLE 3. Radical scavenging activity of each group in percentage.

Groups	Scavenging Activity (%)
Group 1 (CON)	36%
Group 2 (ZIN)	129%*
Group 3 (HFD)	78%*
Group 4 (HFD + ZIN)	125%*

Scavenging Activity (%) of control and treated groups. (1.CON: Control;2. ZIN: Zingerone (100 mg/kg body weight), 3. HFD: (40% of fructose and rodent chow with drinking water (25% fructose), 4. (HFD + ZIN): High fructose diet and zingerone at the dose of 100 mg/kg body weight) \*P<0.001.

#### IV. DISCUSSION

The result showed that there was a significant difference ( $p<0.001$ ) in the DPPH scavenging activity and total phenolic content (TPC) between the control and treated groups. The scavenging activity was found to be highest (128.95%) for Group 2 in which the rats were fed with normal pellet and also given zingerone as the antioxidant compound. For the phenolic content assay, Group 2 showed the highest concentration of 4.44 mg GAE. Both assays showed that Group 2 was having the highest value to indicate the potential of zingerone as antioxidant as well as to prevent the progression of NAFLD to NASH. The gingerone treated high fructose diet rats group (Group 4) also indicated a significant radical scavenging activity. As indicated in recent studies zingerone could be considered in the prevention of NAFLD as well as the worse progression of the disease to NASH. It stated those that having NAFLD could benefit from zingerone as the prevention for the disease<sup>8</sup>. Another study have shown that ginger have the preventive effect for hyperlipidemia which was one of the risk factor for NASH, ginger is one of the top five antioxidant food and one of the alternatives for the preventive effect for hyperlipidemia<sup>9</sup>. Ginger as the supplement for NAFLD can prevent the biochemical abnormalities involving the pathogenesis of NAFLD<sup>5</sup>. In the past study, they characterized the changes in the liver pathology, hepatic lipid composition, and hepatic iron concentration (HIC) in rats' model fed with high fructose diet<sup>10</sup>. The gingerone treated high fructose diet rats group (Group 4) also indicated a significant radical scavenging activity and high phenolic content as indicated by DPPH assay. Phenolics serve in defence by scavenging or preventing the formation of reactive oxygen species (ROS) to avoid molecular damage and other damaging factors<sup>11,12</sup>. (DPPH•) scavenging is one of the assay that are used for determination of scavenging activity which involve in the mechanism of electron transfer<sup>13,14</sup>.

#### V. CONCLUSION

Based on the data gained from this study, zingerone has radical scavenging activity as proven by the DPPH scavenging assay and total phenolic assay. Hence, zingerone may have

antioxidant potential against high fructose diet induced non-alcoholic steatohepatitis . Hence further studies are needed confirm the mechanism of the antioxidant potential of gingerone.

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