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Effect of Local Ginger (*Zingiber officinale*) Extract on S Bilirubin, S. ALT and S. ALP Level on Hepatotoxicity Induced by Paracetamol in Wister Albino Male Rats in Bangladesh

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Abstract

Background: The key organ of metabolism is liver. It can be damaged by prolonged use of drugs like paracetamol. Ginger is a commonly used spice which may have hepatoprotective effect.

Objective: To assess effect of ginger on S. ALT and S. ALP paracetamol induced hepatotoxicity.

Methods: This study was carried out at SSMC, Dhaka on 2022. 24 Wister albino male rats were divided into group A (negative control), group B (positive control) and group C (experimental group); 8 rats in each group. Group B received paracetamol (2 g/kg bw/day) for last 3 days. Group C received ginger (400 mg/kg bw/day) for 28 days and paracetamol for last 3 days. Blood was collected for estimation of bilirubin, ALT and ALP at BSMMU. Statistical analysis was done by using SPSS 23. Paired 't' test, One way ANOVA and post hoc Bonferroni test were done. p value ≤ 0.05 was considered as level of significance.

Result: Mean serum bilirubin, ALT and ALP levels were significantly ($p < 0.001$) higher in positive control group than negative control. Mean serum bilirubin, ALT and ALP level were non-significantly higher in experimental group than negative control. Moreover, mean serum total bilirubin and ALT levels were significantly ($p < 0.001$) lower whereas ALP level was non-significantly lower in experimental group than those of positive control group.

Conclusion: The present study reveals that ginger has hepatoprotective effect against paracetamol induced hepatotoxicity in Wister albino male rats.

Keywords: *Hepatotoxicity; Paracetamol; Ginger; Hepatoprotective; Wister Albino Male Rat*

Introduction

Liver is a vital organ of the body. It plays a crucial role in metabolism, so it is essential for life. The site of decomposition of erythrocytes and storage of glycogen is liver [1]. Therefore, maintenance of a healthy liver is necessary for wellbeing of an individual [2]. Although there has been tremendous development in the field of medical science, still liver disease is one of the main cause of morbidity and mortality in UK [3]. In Bangladesh about 13.2% patients has been suffering from liver disease [4].

Liver disease is mainly caused by exposure to viruses, toxic chemicals, environmental pollutants and certain drugs such as diclofenac [5], acetaminophen [6] etc. As an antipyretic and analgesic drug paracetamol (acetaminophen) is used worldwide. It is safe when used at therapeutic dose but intentional or unintentional overdose causes hepatotoxicity [7]. Paracetamol is the most common cause of drug induced liver disease necessitating transplantation in the United States [8].

Although some drugs like NAC (N-acetylcysteine) are available in market for hepatoprotection but they have some side effects such as fever, fatigue, depression and cough and the most frequent symptoms were mainly gastrointestinal upsets, with diarrhoea and nausea [9]. Susceptibility of the body to the oxidative damage is determined by balance between the production and scavenging of reactive oxygen species (ROS) or free radicals [10]. This balance is essential for preventing damage caused by oxidative stress [11]. Antioxidant helps in scavenging the free radicals. Commonly applied antioxidants are synthetic phenols, such as, butylated hydroxytoluene and butylated hydroxyanisole (BHA). However, their safety is doubtful [12]. Therefore, the use of natural antioxidants has come to the limelight.

Ginger is the rhizomes of the plant *Zingiber officinale* (Family Zingiberaceae) which has been used as one of the most popular culinary agent and spice since ancient time [13]. It is cultivated worldwide. Apart from its culinary use, ginger also possesses medicinal properties and has been used historically in common cold, headache and nausea. Studies have revealed that the unique culinary and medicinal properties of ginger is due to presence of some phytochemicals like zingerone, shogaols, gingerols, α-zingiberene, zingibereno [14]. In abroad preclinical studies carried out with laboratory animals showed that ginger possess hepatoprotective effect to protect the liver against the toxic effects of xenobiotic agents like acetaminophen, CCl_4 [15], heavy metals like mercury [16] and lead [17].

Objectives of the Study

General objective

To assess the hepatoprotective effect of ginger (*Zingiber officinale*) on paracetamol induced hepatotoxicity in Wister albino male rats.

Specific objectives

- To estimate serum levels of total bilirubin, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in experimental group.
- To perform all these above mentioned parameters in negative control and positive control groups for comparison.

Materials and Methods

Study design: Experimental study (Animal study).

Study area: Department of Physiology, Sir Salimullah Medical College, Dhaka.

Study population: Twenty four (24) Wister albino male rats.

Study period: 1st July 2021 to 30th June 2022.

Research subjects: Wister albino male rats.

Inclusion criteria: Apparently healthy Wister albino male rats 90 to 120 days old weighing between 200 to 250 grams.

Exclusion criteria: Unhealthy or diseased rats.

Statistical analysis: SPSS.

Ethical approval: Protocol was approved by Institutional Ethics Committee (IEC) of Sir Salimullah Medical College (SSMC), Dhaka.

Grouping of the rats: After acclimatization for 14 days, all 24 rats were divided into three groups.

1. **Group A - Negative control group:** Consisted of eight (8) Wister albino male rats. They received basal diet orally for 28 days.
2. **Group B - Positive control group (Paracetamol treated control group):** Consisted of eight (8) Wister albino male rats. In addition to basal diet they received paracetamol orally (2 g/kg bw/day) for last 3 days (26th to 28th days) of study period.

3. **Group C - Experimental group (Ginger extract pretreated and paracetamol treated group):** Consisted of eight (8) Wister albino male rats. In addition to basal diet, they received ginger extract orally (400 mg/kg bw/day) for 28 days and paracetamol orally (2 g/kg bw/day) for last 3 days (26th to 28th) of study period.

Dose and duration

Paracetamol:

- **Dose:** 2 g/kg body weight orally by gastric gavage [18].
- **Duration:** For last 3 days (26th to 28th) of study period.

Ginger extract:

- **Dose:** 400 mg/kg body weight orally by gastric gavage.
- **Duration:** Daily in the morning between 9.00 AM to 10.00 AM for twenty eight consecutive days [19].

Study procedure

According to selection criteria all the animals were purchased from animal house of BSMMU, Dhaka and the animals were kept in the animal house of Institute of Nutrition and Food Science, University of Dhaka, where the experiment was carried out. All the rats were acclimatized for 14 days prior to intervention at 27 to 28˚C room temperature [20]. They were kept under 12 hours dark - light cycle with free access to standard rat pellet diet and allowed drinking water as desired. All rat's body weight were measured at the beginning and at the end of study period. The serum level of alanine aminotransferase (ALT) was measured from tail vein of rats on day 1 and rats with normal serum level of ALT were included in this experiment.

All the rats received basal diet for 28 days. Experimental group (group C) received aqueous extract of ginger 400 mg/kg body weight in the morning between 9:00 AM to 10:00 AM for 28 days. Hepatotoxicity was induced by single daily morning dose of paracetamol 2 g/kg body weight orally by gastric gavage on 26th, 27th and 28th day after overnight fasting in positive control group (group B) and experimental group (group C). At the end of the study period on (29th day), all the rats (24) were sacrificed and blood sample was collected for estimation of all the biochemical parameters.

Result

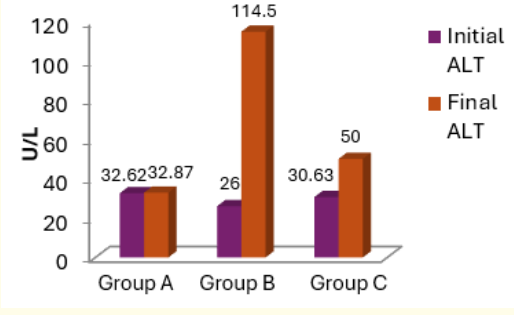

Figure 1: *Mean initial and final serum ALT levels in different groups of rats (N = 24).*

Group A: Negative control group.

Group B: Positive control group (paracetamol treated control group).

Group C: Experimental group (ginger extract pretreated and paracetamol treated group).

N = Total number of rats; n = Number of rats in each group.

Group	Serum ALT (U/L)		p-value
	Initial (I)	Final (F)	
Group A (n = 08)	32.62 ± 4.10 (27 - 39)	32.88 ± 4.02 (27 - 39)	0.17^{ns}
Group B (n = 08)	26.00 ± 3.74 (21 - 32)	114.50 ± 29.17 (77 - 152)	< 0.001***
Group C (n = 08)	30.63 ± 5.83 (24 - 39)	50.00 ± 3.74 (45 - 57)	< 0.01**

Table 1: *Comparison between mean initial and final serum ALT levels in different groups of rats (N = 24).*

Data are expressed as mean ± SD. For statistical analysis, paired 't' test was done to compare initial and final ALT in same group. Figures in parentheses indicate ranges.

Result shown in figure 1 and table 1

The mean (± SD) initial serum ALT levels were 32.62 ± 4.10, 26.00 ± 3.74 and 30.63 ± 5.83 U/L, mean final serum ALT levels were 32.88 ± 4.02, 114.50 ± 29.17 and 50.00 ± 3.74 U/L, in group A, group B and group C respectively.

The mean (± SD) initial and final serum ALT levels were almost similar in group A and the differences were statistically non-significant. The mean (± SD) final serum ALT level was significantly higher in group B ($p < 0.001$) and group C ($p < 0.01$) in comparison to that of initial ALT level of both groups respectively.

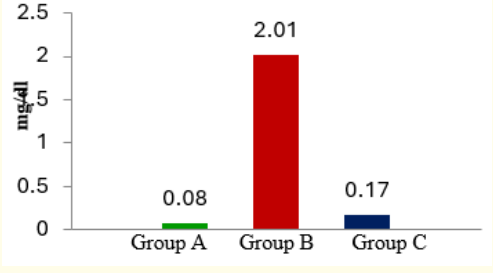

Figure 2: *Mean serum total bilirubin level in different groups of rats (N = 24).*

The mean (± SD) serum total bilirubin level was 0.08 ± 0.03, 2.01 ± 0.34 and 0.17 ± 0.02 mg/dl in group A, B and C respectively (Figure 2).

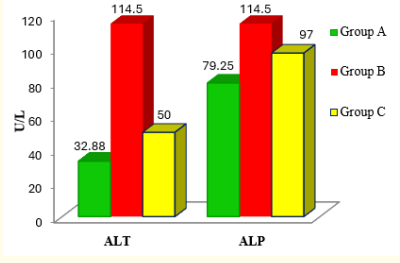

Figure 3: *Mean serum ALT and ALP levels in different groups of rats (N = 24).*

Group A: Negative control group.

Group B: Positive control group (paracetamol treated control group).

Group C: Experimental group (ginger extract pretreated and paracetamol treated group).

N = Total number of rats; n = Number of rats in each group.

Result shown in figure 3

Mean serum ALT level was 32.88 ± 4.02, 114.50 ± 29.17 and 50.00 ± 3.74 U/L, mean serum AST level was 46.38 ± 8.33, 220.50 ± 17.55 and 120.63 ± 28.45 U/L, in group A, B and C respectively.

Group	Serum Total bilirubin	Serum ALT	Serum ALP
	p-value	p-value	p-value
A vs B vs C	<0.001***	<0.001***	<0.01**
A vs B	<0.001***	<0.001***	<0.01**
A vs C	1.000^{ns}	0.176^{ns}	0.234^{ns}
B vs C	<0.001***	<0.001***	0.246^{ns}

***Table 2**: Multiple comparisons.*

Data are expressed as mean ± SD. For statistical analysis, one way ANOVA test was performed for comparison among the groups and then post hoc-Bonferroni test to compare between groups. Figures in parentheses indicate ranges. N = Total number of rats; n = Number of rats in each group; ns = Non significant.

The results are shown in table 2

The mean (± SD) serum total bilirubin level was significantly ($p < 0.001$) higher in group B in comparison to that of group A, whereas this level was significantly ($p < 0.001$) lower in group C than that of group B. Serum total bilirubin level in group A and group C was almost similar and the difference was not statistically significant

The mean (± SD) serum ALT level was significantly ($p < 0.001$) higher in group B in comparison to that of group A, whereas ALT level was significantly ($p < 0.001$) lower in group C than that of group B. Again serum ALT level in group C was higher in comparison to that of group A although the difference was not statistically significant.

The mean (± SD) serum ALP level was significantly ($p < 0.01$) higher in group B in comparison to that of group A. Again this level was also higher in group C in comparison to that of group A but the difference was not statistically significant. Whereas this level was lower in group C in comparison to that of group B but the difference was not statistically significant.

Discussion

The present study was carried out to evaluate the hepatoprotective effect of ginger on paracetamol induced hepatotoxic rats. For the purpose of the study serum levels of total bilirubin, alanine aminotransferase (ALT) and alkaline phosphate (ALP) to assess liver function.

Serum total bilirubin

In this study, serum total bilirubin level was significantly ($p < 0.001$) higher in positive control group in comparison to that of negative control group. Similar finding was also observed by Yemitan [15] and Fadil, H., *et al*. [21].

Again, serum total bilirubin level was significantly ($p < 0.001$) lower in experimental group than that of positive control group. Similar finding was observed by Yassin., *et al*. [22].

Serum alanine aminotransferase (ALT)

In this study, serum ALT level was significantly ($p < 0.001$) higher in positive control group than negative control group. Similar finding was observed by investigators of various countries Yemitan [15], Yassin., *et al*. [22], Hamid., *et al*. [23], Pinho., *et al*. [24], Abdel-Azeem, Amal S., *et al*. [25], Fakurazi., *et al*. [26].

Furthermore, serum ALT level was significantly ($p < 0.001$) lower in experimental group (ginger extract pretreated and paracetamol treated group) than that of positive control group. Similar observation was made by Aborehab [19], Fadil., *et al*. [21], Hemabarath., *et al*. [27], Bakr [28].

Serum alkaline phosphatase (ALP)

In this study, serum ALP level was significantly ($p < 0.01$) higher in positive control group in comparison to that of negative control group. Almost similar finding was also observed by Yemitan [15], Yassin., *et al*. [22], Abdel-Azeem., *et al*. [25], Ajith., *et al*. [29].

Furthermore, serum ALP level was lower in experimental group in comparison to that of positive control group although it was not statistically significant. This finding was also in agreement to that of Fakurazi., *et al*. [26]. Whereas significant reduction of serum ALP level in experimental group in comparison to that of positive control group observed by Fadil., *et al*. [21], Abdel-Azeem, Amal S., *et al*. [25].

On the contrary, significant elevation of serum ALP level in experimental group in comparison to that of positive control group observed by Lebda., *et al*. [30]. This discrepancy was may be due to prolong duration of administration of paracetamol for 21 days (3 weeks).

In the present study, paracetamol induced hepatotoxicity was observed in Wister albino male rats as evidenced by their measured higher serum levels of total bilirubin, ALT and ALP concentration in liver. These changes might be due to increased production of free radicals which initiate lipid peroxidation and subsequent cellular damage.

Again, Mean serum total bilirubin, ALT levels were significantly ($p < 0.001$) lower in experimental group suggested the possibility of the ginger extract having hepatoprotective effect against paracetamol induced liver injury. However, ALP level was non-significantly lower in experimental group than those of positive control group, this might be due to Alkaline phosphatase (ALP) is mainly secreted from biliary tract. That might be the reason of non significant lower ALP level in experimental group after giving ginger extract. Nevertheless, the exact mechanism involved can not be elucidated from this study due to time and financial constraints.

Conclusion

From this study it is concluded that ginger has hepatoprotective effect on paracetamol induced hepatotoxicity in Wister albino male rats.

Limitation of the Study

- Different doses of ginger extract were not used to find out the best effective dose.
- Anti-oxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and succinate dehydrogenase (SDH) levels were not studied.

Recommendation

For further study following recommendation are proposed:

- Similar type of study with different doses of ginger and with different compounds of ginger extract.
- Estimation of anti-oxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), succinate dehydrogenase (SDH) levels etc.
- Evaluation of chronic model of hepatotoxicity.

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