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Original Article

Effect of Green Tea (Camellia Sinensis) on Paracetamol Induced Liver Damage In Long Evans Male Rats

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Abstract:

Background: Liver is an essential organ of our body that can be damaged by poisonous effects of chemicals, toxins, prolonged and uncontrolled use of drugs. Green tea is a popular beverage which may have hepatoprotective effect.

Objective: To observe the effect of green tea (Camellia sinensis) on paracetamol induced liver damage in Long Evans male rats.

Methods: This study was carried out in the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka from July 2018 to June 2019. A total number of thirty (30) apparently healthy Long Evans male rats, 90-120 days old, weighing between 150-200g was taken for the study. After acclimatization for 14 days, they were divided into two groups, control group (Group A) and experimental group (Group B – green tea pretreated and paracetamol treated group). Control group was subdivided into group A1 (baseline control group) and group A2 (paracetamol treated control group). Each of this group was consisted of ten rats. All the rats received basal diet for 28 days. In addition to basal diet, baseline control group also received normal saline (20 ml/kg/day) orally daily for 28 days. Paracetamol treated control group received paracetamol orally (1.5 g/kg/day) for last 3 days (26th to 28th days) of the study period. Again experimental group received ethanolic extract of green tea orally (500 mg/kg/day) for 28 days and paracetamol orally (1.5g/kg/day) for last 3 days (26th to 28th days) of the study period. All the rats were sacrificed on 29th day. After sacrifice blood and liver sample was collected. Blood sample was collected from heart. Serum levels of total bilirubin, ALT and ALP were measured.

One way ANOVA test, post hoc-Bonferroni test and Chi-square test were done to compare the data as applicable.

Result: The mean serum total bilirubin, ALT and ALP levels were significantly higher in paracetamol treated control group and green tea pretreated and paracetamol treated group in comparison to those of baseline control group. Again, mean serum total bilirubin, ALT and ALP levels were significantly lower in green tea pretreated and paracetamol treated group than those of paracetamol treated control group.

Conclusion: This study revealed that green tea has hepatoprotective effect against paracetamol induced liver damage in Long Evans male rats.

Keywords: Green Tea, Liver Damage, Long Evans Male Rats.

Introduction

Liver is the largest organ in the body. It is essential for life for it's many biochemical and metabolic functions. Among many complex functions of liver, production and secretion of bile, nutrient and vitamin metabolism, detoxification of drugs and toxins, synthesis of clotting factors and plasma proteins are the most important.[1]

Liver disease is very common worldwide and is one of the leading causes of mortality. The continuous exposure to some factors like virus, alcohol, fatty diet and bio transformed metabolites can cause liver injury leading to inflammation and liver degeneration. Liver damage can also lead to steatosis, steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma.[2] Severe acute liver disease may lead to fulminant or acute liver failure (Anstee and Jones, 2018). In Bangladesh, about 13.2% patients visiting hospital OPD are suffering from liver diseases (Rahman et al. 2014). Among the liver diseases fulminant hepatic failure has the worst prognosis with a mortality rate of around 73.1%.[3] Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may cause of liver injury. Reactive oxygen species are involved in liver damage caused by several conditions such as alcohol abuse, fibrosis/cirrhosis of various etiologies, hepatocellular carcinoma, paracetamol overdose and viral hepatitis.[4] Drug induced liver injury is a common adverse event encountered in clinical practice. Paracetamol is a widely used antipyretic and analgesic drug which is used for treatment of fever, headache and other pains. Despite having beneficial effects misuse of paracetamol through uptake of supratherapeutic doses may lead to hepatic, renal[4] and brain adverse side effects in humans and experimental animals.[5]

Paracetamol is a widely used drug to induce hepatic damage in experimental models. Acute liver failure caused by paracetamol is due to the metabolic activation of this drug to a toxic metabolite named N-acetyl-p-benzoquinone imine (NAPQI) in the liver by cytochrome p450 isoenzyme especially CYP2E1. It is known that NAPQI depletes liver glutathione thereby induces oxidative stress.[6] As a result excess production of reactive oxygen species (ROS) like superoxide radicals, hydrogen radicals and hydroxyl radicals may invade the biological molecules such as DNA, protein, phospholipids etc. It also may lead to lipid peroxidation and depletion of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).[7] On the basis of these facts researchers across the world are now showing interest in the use of alternative medicines for the treatment of hepatic disease. Natural products received great attention as they are potentially antitoxic and antioxidants agents.[8]

Green tea is a popular beverage across the globe. Polyphenols found in green tea are proven antioxidant and anti-inflammatory agent.[9] Green tea is produced from the dried leaves of the plant Camellia sinensisand contains several polyphenolic compounds. Most of these polyphenols are flavonols. They are usually called catechins. Epigallocatechingallate (EGCG) is the most abundant catechin, comprising for about 65% of green tea catechin content. It is also the component with the highest antioxidant properties. Oxidative stress due to generation of reactive oxygen species can be prevented by catechins. The protective effect of green tea is due to the ability of its catechins to prevent the production of oxygen radicals and also scavenge free radicals such as peroxyl, hydroxyl and superoxide anions.[10] Many previous journals also documented many other beneficial effects of green tea such as anti-obesity, anticarcinogenic,[11] hypocholesterolemic,[12] anti-neurodegenerative[13] properties. Green tea also protects against MTX,[14] cyclophosphamide,[15] Tamoxifen,[16] Azathioprine[17] induced hepatotoxicity. Green tea extract is considered to be safe for clinical use in many studies.[18] Green tea also has preventive action on paracetamol induced hepatotoxicity in Long Evans rats.[19]

Materials and methods

This Experimental study was conducted in the Department of Physiology, Sir Salimullah Medical College, Dhaka from 1st July 2018 to 30th June 2019. The study was approved by Institutional Ethics Committee of SSMC. Purposive sampling followed by randomization was done. A total number of thirty (30) apparently healthy Long Evans male rats, 90 to 120 days old were selected on the basis of inclusion and exclusion criteria. Rats were purchased from animal house of Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU).

Grouping of the rats:

After acclimatization for 14 days all the animals were divided into 2 groups after randomization, Group: A (Control group) and Group: B (Experimental group).

Group A: Control group

Consisted of 20 rats. This group was subdivided into group A_1 and A_2.

• **Group A_1:** baseline control group consisted of ten (10) rats. In addition to basal diet they received normal saline (20ml/kg/day) orally for 28 days.

• **Group A_2:** paracetamol treated control group consisted of ten (10) rats. In addition to basal diet they received paracetamol orally (1.5g/kg/day) for last 3 days (26th to 28th days) of study period.

Group B: Experimental group:
Green tea pretreated and paracetamol treated group

• Consisted of ten (10) rats. In addition to basal diet they received ethanolic extract of green tea orally (500mg/kg/day) for 28 days and paracetamol orally (1.5g/kg/day) for last 3 days (26th to 28th days) of study period.

Doses and duration:
Paracetamol
• **Dose:** 1.5g/kg body weight orally by gastric gavage.

• **Duration:** Daily in the morning between 09:00 AM to 10:00 AM for last 3 days (26th to 28th days) of study period.

Green tea

• **Dose:** 500 mg/kg body weight orally by gastric gavage.

• **Duration:** Daily in the morning for twenty eight (28) consecutive days (from day 1 to day 28).

According to selection criteria all the rats were purchased from animal house of Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. They were kept in the animal house of Institution of Nutrition and Food Science, University of Dhaka where the experiment was carried out. All the animals were acclimatized for 14 days prior to intervention at 23±2 ºC room temperature under 12 hours light/12 hours dark cycle. During this period, the animals had free access to standard food pellets and allowed drinking water as desired. After 14 days acclimatization the total study period was twenty eight (28) days. At the beginning of the study period (day 1) initial body weight of all the rats were measured and at the end of the study period their final body weight were measured. Blood samples were collected on day-1 from the tail vein of all rats to assess the liver function. The serum level of alanine aminotransferase (ALT) was measured and rats with normal level of ALT (10-40 U/L) were included in this experiment.

All the rats received basal diet. In addition to basal diet, rats of baseline control group received normal saline (20 ml/kg body weight) orally daily. Hepatotoxicity was induced by administration of single daily morning dose of paracetamol (1.5g/kg body weight) orally by gastric gavage on day 26, 27 and 28 after overnight fasting in all groups of rats except baseline control group. Green tea extract (500 mg/kg/day dissolved in 1ml distilled water) was given to experimental group (group B) orally in the morning between 9:00 AM to 10:00AM for 28 consecutive days.

At the end of the study period, all rats were sacrificed on day 29 (after 24 hours of last dose of paracetamol administration on day 28). They were anesthetized with the help of chloroform (30%). Then blood samples (5ml) were collected from the heart by using disposable syringe and were taken in separate clean and dry test tubes with proper identification numbers and were kept in standing position till formation of clot. Then blood was centrifuged at a rate of 3000 rpm for 10 minutes. After that supernatant serum were collected in labeled eppendroff tube and preserved in the refrigerator for estimation of all the biochemical parameters. Liver was also removed from each rat and weighed. Serum levels of total bilirubin, alanine aminotransferase (ALT) and alkaline phosphates (ALP) were measured in Department of Physiology, SSMC by semi automated analyzer machine. Statistical analysis was done by using Statistical Package for Social Science (SPSS) for windows version 22. Data were expressed as mean ± SD. For statistical analysis, ANOVA, post hoc-Bonferroni test and Chi-square test were done as applicable. p value ≤0.05 was considered statistically significant.

Results
Serum total bilirubin, ALT and ALP levels in different groups of rats (N=30) are shown in table-I

Table-I shows that the mean (±SD) serum total bilirubin level was significantly higher in group A_2 ($p<0.001$) and B ($p<0.05$) in comparison to that of group A_1, whereas this level was significantly ($p<0.001$) lower in group B than that of group A_2. On the other hand, the mean (±SD) serum ALT was significantly higher in group A_2 and B ($p<0.001$ and $p<0.01$ respectively) in comparison to that of group A_1, whereas ALT level was significantly ($p<0.001$) lower in group B than that of group A_2.

Again, the mean (±SD) serum ALP was significantly ($p<0.001$) higher in group A_2 in comparison to that of group A_1, whereas ALP level was significantly ($p<0.001$) lower in group B than that of group A_2. ALP level in group A_1 and group B was almost similar and the difference was not statistically significant (table-I).

Table-I: Serum total bilirubin, ALT and ALP level in different groups of rats (N=30)

Group	Serum Total Bilirubin (mg/dl)	Serum ALT (U/L)	Serum ALP (U/L)
A_1 (n=10)	0.67 ± 0.27	37.00 ± 3.37	88.60 ± 11.63
	(0.25 - 1.05)	(31 - 42)	(73 - 110)
A_2 (n=10)	2.42 ± 0.52	83.20 ± 17.50	141.30 ± 6.41
	(1.72 - 3.33)	(66 - 118)	(133 - 151)
B (n=10)	1.17 ± 0.20	53.50 ± 6.36	89.80 ± 6.81
	(0.87 - 1.52)	(46 - 64)	(79 - 100)

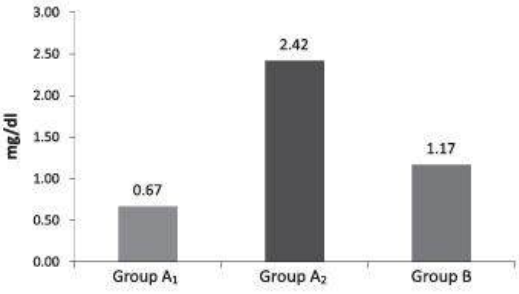
Multiple comparison

	Serum Total bilirubin	Serum ALT	Serum ALP
	p-value	p-value	p-value
A_1vs A_2vs B	0.000***	0.000***	0.000***
A_1vs A_2	0.000***	0.000***	0.000***
A_1vs B	0.013*	0.007**	1.000ns
A_2vs B	0.000***	0.000***	0.000***

Data are expressed as mean ± SD. For statistical analysis, ANOVA test was performed for comparison among the groups and then post hoc-Bonferroni test to compare between groups. Figures in parentheses indicate ranges.

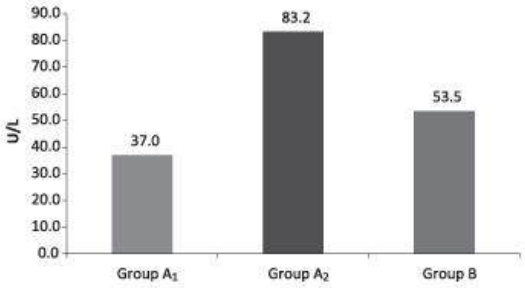
Group A_1: Baseline control group, **Group A_2:** Paracetamol treated control group, **Group B:** Experimental group (green tea pretreated and paracetamol treated group), N=Total number of rats; n= number of rats in each group; ns = not significant; *=significant at p-value < 0.05; **=significant at p-value< 0.01; *** = significant at p-value< 0.001

Figure-I: Mean serum total bilirubin level in different groups of rats (N=30)



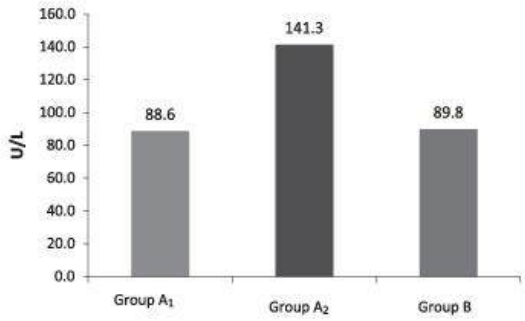
Group A_1: Baseline control group, **Group A_2:** Paracetamol treated control group, **Group B:** Experimental group (green tea pretreated and paracetamol treated group), N = Total Number of rats

Figure-II: Mean serum ALT level in different groups of rats (N=30)



Group A_1: Baseline control group, **Group A_2:** Paracetamol treated control group, **Group B:** Experimental group (green tea pretreated and paracetamol treated group), N = Total Number of rats

Figure-III: Mean serum ALP level in different groups of rats (N=30)



Group A_1: Baseline control group, **Group A_2:** Paracetamol treated control group, **Group B:** Experimental group (green tea pretreated and paracetamol treated group), N = Total Number of rats

DISCUSSION

The present study was carried out to evaluate the hepatoprotective effect of green tea on paracetamol induced hepatotoxic rats. For the purpose of the study serum levels of total bilirubin, alanine aminotranferase (ALT), alkaline phosphatase (ALP), malondialdehyde (MDA) in liver homogenate were measured to assess liver function. Moreover, histological examination of liver was also done to observe the microscopical findings of the liver. In the present study, thirty (30) Long Evans male rats, 90 to 120 days old, weighing between 150-200 grams were taken. After acclimatization for 14

days, all the rats were randomly divided into three (3) groups such as baseline control group (A_1), paracetamol treated control group (A_2) and green tea pretreated and paracetamol treated group (B). All groups of rats received basal diet for 28 days. To produce hepatotoxicity, paracetamol treated control group (A_2) and green tea pretreated and paracetamol treated group (B) were given paracetamol orally (1.5g/kg/day) for last 3 days (26th to 28th days) of study period. In addition to this, green tea pretreated and paracetamol treated group (B) also received ethanolic extract of green tea orally (500mg/kg/day) for 28 days (day 1 to day 28).

In this study, values of the study parameters such as serum total bilirubin, ALT, ALP levels of all the rats of baseline control group were within physiological limit and the histological findings of liver revealed normal histological architecture. These findings were almost similar to those reported by various researchers of different countries.[19]

In this study, serum total bilirubin level was significantly higher in paracetamol treated control group ($p<0.001$) and green tea pretreated and paracetamol treated group ($p<0.05$) in comparison to that of baseline control group. Similar finding was also observed by Lodhi[20] and Abolfathi.[21]

Again serum total bilirubin level was significantly ($p<0.001$) lower in green tea pretreated and paracetamol treated group than that of paracetamol treated control group. Almost similar finding was observed by different researchers Adel[22] and Hamden.[23]

In this study, serum ALT was significantly ($p<0.001$) higher in paracetamol treated control group and green tea pretreated and paracetamol treated group ($p<0.001$ and $p<0.01$ respectively) in comparison to that of baseline control group. Almost similar finding was observed by Saad,[24] Deib and Ahmed.[25]

Again serum ALT level was significantly ($p<0.001$) lower in green tea pretreated and paracetamol treated group than that of paracetamol treated control group. Almost similar finding was observed by El-kholy[26] and Mehri.[27]

In this study, serum ALP was significantly ($p<0.001$) higher in paracetamol treated control group in comparison to that of baseline control group. Almost similar finding was also observed by Issabeagloo,[28] Jweid[29] and El-Beshbishy.[30]

Again serum ALP was significantly ($p<0.001$) lower in green tea pretreated and paracetamol treated group than that of paracetamol treated control group. Almost similar finding was observed by Mohammed.[31]

Conclusion

In this study it was observed that green tea has hepatoprotective effect against paracetamol induced liver damage in Long Evans male rats.

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