

Basic Research

Hepatocellular Changes on Paracetamol Induced Liver Damage in Long Evans Male Rats upon Green Tea Administration

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Abstract:

Background: The liver is a vital organ that serves a number of purposes in our body. It may be harmed by pollutants, substances with toxic effects, and long-term, unchecked drug use. Green tea is a well-liked beverage that has gained popularity recently and may have hepatoprotective properties.

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

Methods: From July 1st, 2018, to June 30th, 2019, this study was conducted in the Department of Physiology at Sir Salimullah Medical College (SSMC), Dhaka. For the purpose of the study, thirty (30) male Long Evans rats, weighing between 150 and 200 grams and 90 to 120 days old, appeared to be in good health. They were split into two groups after 14 days of acclimatization: Group A, which served as the control group, and Group B, which served as the experimental group (green tea pretreatment and paracetamol treated). Group A1 (baseline control group) and group A2 (paracetamol treated control group) comprised the subdivided control group. There were ten rats in each of these groups. For 28 days, the rats were all fed a baseline diet. The baseline control group was given normal saline (20 ml/kg/day) orally every day for 28 days in addition to their basal diet. For the final three days of the study—the 26th and 28th—the paracetamol-treated control group was given oral paracetamol at a dose of 1.5 g/kg/day. During the final three days of the study period (the 26th and 28th days), the experimental group was given oral paracetamol (1.5g/kg/day) and an ethanolic extract of green tea (500 mg/kg/day) for a total of 28 days. On day 29, every rat was sacrificed. Following the sacrifice, liver samples were taken. Hepatic contents of malondialdehyde (MDA) were measured by using standard laboratory method. Histopathology of liver was also done by using standard laboratory procedure in the department of pathology, SSMC. Version 22 of the Statistical Package of Social Science (SPSS) for Windows was used to do the statistical analysis. The data were displayed as mean±SD. To compare the results, one-way ANOVA, post hoc Bonferroni, and Chi-square tests were used, where appropriate. A p value of less than 0.05 was regarded as significant.

Result: When compared to the baseline control group, the mean malondialdehyde concentration in the liver was significantly (p) greater in the green tea pretreatment, paracetamol treated, and control groups who received paracetamol treatment. Once more, the groups treated with green tea and paracetamol had mean malondialdehyde concentrations in their livers that were significantly (p) lower than those of the paracetamol group. Additionally, 0% of the rats in the baseline control group, 100% of the rats in the paracetamol-treated group, and 30% of the rats in the green tea-pretreated and paracetamol-treated group had abnormal histological findings of the liver.

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

Introduction:

Within the body, the liver is the largest and most important gland. Due to its numerous biochemical and metabolic roles, it is necessary for life. The liver's numerous intricate processes include the creation of plasma proteins and clotting factors, the secretion and generation of bile, the metabolism of nutrients and vitamins, the detoxification of medications and poisons, and much more.¹

One of the main causes of death worldwide is liver disease, which is also highly prevalent. Liver injury can result in inflammation and liver degeneration when certain variables, such as viruses, alcohol, fat diets, and biotransformed metabolites, are continuously exposed to the liver. Steatosis, steatohepatitis, fibrosis, cirrhosis, and hepatocellular cancer

can also result from liver injury.²

Acute or fulminant liver failure can result from severe acute liver disease. According to Rahman et al. (2014), 13.2% of patients in Bangladesh who attend the hospital's outpatient department have liver disorders. With a death rate of roughly 73.1%, fulminant hepatic failure has the worst prognosis among liver illnesses.³

Liver damage can occur from some medications when taken in excess or occasionally even when prescribed within therapeutic parameters. Liver damage resulting from multiple disorders, including alcohol abuse, fibrosis/cirrhosis of different etiologies, hepatocellular carcinoma, overdosing on paracetamol, and viral hepatitis, is associated with reactive oxygen species.⁴

Despite having beneficial effects misuse of paracetamol through uptake of supratherapeutic doses may lead to hepatic, renal⁴ and brain adverse side effects in humans and experimental animals.⁵

One common medication used to cause liver injury in experimental models is paracetamol. The cytochrome p450 isoenzyme, particularly CYP2E1, metabolically activates paracetamol to produce the poisonous metabolite N-acetyl-p-benzoquinone imine (NAPQI) in the liver, which causes acute liver failure. It is well known that NAPQI causes oxidative stress by depleting liver glutathione.⁶

Reactive oxygen species (ROS) such as superoxide radicals, hydrogen radicals, and hydroxyl radicals may consequently be produced in excess and infiltrate biological components including DNA, proteins, phospholipids, etc. Additionally, it might result in the peroxidation of lipids and the reduction of antioxidant enzymes like glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD).⁷

These factors have led to a global interest in the use of complementary and alternative medicine in the treatment of liver illness among researchers. Natural goods have drawn a lot of interest since they may be antioxidants and antitoxicants.⁸

All across the world, green tea is a popular beverage. Green tea's polyphenols have been shown to have anti-inflammatory and antioxidant properties.⁹ Dried *Camellia sinensis* leaves are used to make green tea, which has a number of polyphenolic substances. These polyphenols are primarily flavonols. Usually, they're referred to as catechins. The most prevalent catechin, epigallocatechin gallate (EGCG), makes up over 65% of the total catechin content in green tea. Additionally, it has the strongest antioxidant qualities of all the components. Catechins have the ability to stop oxidative damage caused by reactive oxygen species production. Green

tea's antioxidant properties stem from its catechins' capacity to scavenge free radicals such superoxide, hydroxyl, and peroxy anions as well as inhibit the generation of oxygen radicals.¹⁰

Numerous scholarly publications have also documented numerous additional advantageous aspects of green tea, including its anti-obesity, anticarcinogenic¹¹, hypocholesterolemic¹², anti-neurodegenerative¹³, and anti-obesity qualities. Additionally, hepatotoxicity caused by MTX¹⁴, cyclophosphamide¹⁵, tamoxifen¹⁶, azathioprine¹⁷ and is prevented by green tea. Numerous investigations have concluded that using green tea extract in a therapeutic setting is safe¹⁸. Furthermore, green tea shields Long Evans rats against the hepatotoxic effects of paracetamol.¹⁹

METHODOLOGY

This experimental study was done from 1st July 2018 to 30th June 2019 in Department of Physiology, Sir Salimullah Medical College, Dhaka. Purposive sampling was done followed by randomization. A total number of thirty (30) Long Evans male rats were included in the study. Based on inclusion and exclusion criteria, thirty (30) male Long Evans rats between the ages of 90 and 120 days were chosen. All of them appeared to be in good health. Rats were purchased from Bangabandhu Sheikh Mujib Medical University's (BSMMU) animal house which was maintained by Department of Pharmacology.

Selection Criteria

- **Inclusion criteria:**
 - Long Evans male rats.
 - 90 to 120 days old.
 - Weighing between 150 to 200 grams.
 - Apparently healthy
- **Exclusion Criteria:**
 - Unhealthy diseased rats.

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 A₁ and A₂.

- **Group A₁: baseline control group**
included ten (10) rats in total. They were given normal saline(20 ml/kg/day) orally for 28 days in addition to their regular diet.
- **Group A₂: paracetamol treated control group**
included ten (10) rats in total. For the final three days of the study period (the 26th and 28th days), they were given oral paracetamol (1.5g/kg/day) in addition to their regular diet.

Group B: Experimental group:

Green tea pretreated and paracetamol treated group

- Included ten (10) rats. In addition to their regular diet, they were given 500 mg/kg of ethanolic green tea extract

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orally every day for 28 days, and 1.5 mg/kg of paracetamol orally every day for the final three days of the study period (the 26th and 28th days).

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 (because other researchers also followed similar food schedule as if paracetamol given earlier for 3 consecutive days, the rats would have died earlier, hence the result would not be as expected)

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).

Study procedure:

All of the rats were acquired from the animal house of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, in accordance with the selection criteria. The experiment was conducted in the animal house of the University of Dhaka's Institution of Nutrition and Food Science. Prior to intervention, all animals were acclimated for 14 days at room temperature, 23±2 °C, with a light/dark cycle of 12 hours each day. The animals were given free access to regular meal pellets and were permitted to drink as much water as they pleased during this time. Following 14 days of acclimatization, the study period lasted a total of 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.

The rats were fed a baseline diet. Normal saline (20 ml/kg body weight) was given orally to the rats in the baseline control group every day in addition to their basal diet. With the exception of the baseline control group, all groups of rats experienced hepatotoxicity when a single daily morning dose of paracetamol (1.5g/kg body weight) was administered orally by gastric gavage on days 26, 27, and 28 following an overnight fast. The experimental group (group B) received green tea extract orally in the morning between 9:00 AM and 10:00 AM for 28 days in a row (500 mg/kg/day diluted in 1 ml distilled water).

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%). Liver was also removed from each rat and weighed. Assessment of malondialdehyde (MDA) content of liver tissue homogenate was done by using standard laboratory kits in the laboratory of Department of Biochemistry and Molecular Biology, Jahangir Nagar University, Savar, Dhaka. To find out the histopathological changes of liver tissue, histological slides were prepared, observed under microscope and photomicrographs were taken by using standard laboratory procedure in the Department of Pathology, SSMC.

Statistical analysis:

Statistical analysis was done 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:

Body weight and liver weight of different groups of rats (N=30)

The results are shown in Table I and Figure 1, 2.

The mean (±SD) initial body weight of rats on day-1 were 175.60 ± 12.17, 184.90 ± 13.54 and 176.80 ± 14.20 g, whereas the final body weight on day-28 were 210.20 ± 7.97, 210.80 ± 7.69 and 217.70 ± 13.19 g in group A1, A2 and B respectively. The liver weight of rats was 3.19 ± 0.12, 4.98 ± 0.44 and 4.20 ± 0.61 g in group A1, A2 and B respectively.

Initial body weight of group A1, A2 and B was almost similar and the difference was not statistically significant.

Also final body weight of group A1, A2 and B was almost similar and the difference was not statistically significant.

The liver weight was significantly (p<0.001) higher in group A2 and B in comparison to group A1. Again liver weight was significantly (p<0.01) lower in group B than that of group A2.

Table I

Body weight and Liver weight in different groups of rats (N=30)

Group	Body weight (g)		% change of body weight from final (F) to initial (I) [(F-I)/Ix100]	Liver Weight (g)
	Initial (I)	Final (F)		
A1 (n=10)	175.60 ± 12.17 (152 - 190)	210.20 ± 7.97 (200 - 226)	20.14 ± 8.17 (7.89 - 33.55)	3.19 ± 0.12 (3.08 - 3.38)
A2 (n=10)	184.90 ± 13.54 (166 - 204)	210.80 ± 7.69 (202 - 222)	14.43 ± 7.41 (2.94 - 27.11)	4.98 ± 0.44 (4.23 - 5.67)
B (n=10)	176.80 ± 14.20 (160 - 198)	217.70 ± 13.19 (201 - 241)	23.65 ± 9.86 (5.08 - 39.75)	4.20 ± 0.61 (3.73 - 5.51)

Multiple comparison

	Initial body weight	Final body weight	% change of body weight	Liver weight
	p value	p value	p value	p value
A1 vs A2 vs B	0.254 ^{ns}	0.192 ^{ns}	0.069 ^{ns}	0.000 ^{***}
A1 vs A2	0.391 ^{ns}	1.000 ^{ns}	0.440 ^{ns}	0.000 ^{***}
A1 vs B	1.000 ^{ns}	0.310 ^{ns}	1.000 ^{ns}	0.000 ^{***}
A2 vs B	0.556 ^{ns}	0.397 ^{ns}	0.069 ^{ns}	0.002 ^{**}

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.

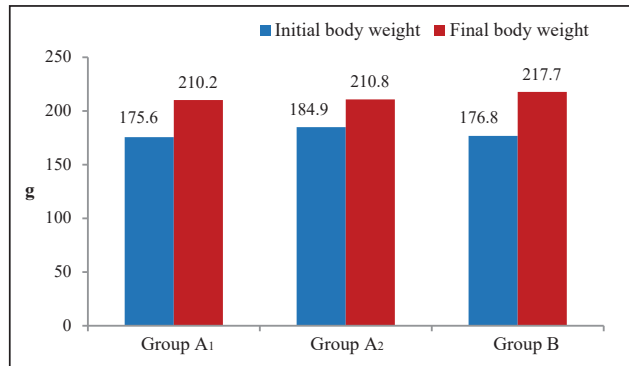
Group A1: Baseline control group

Group A2: 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< 0.01; * = significant at p-value< 0.001



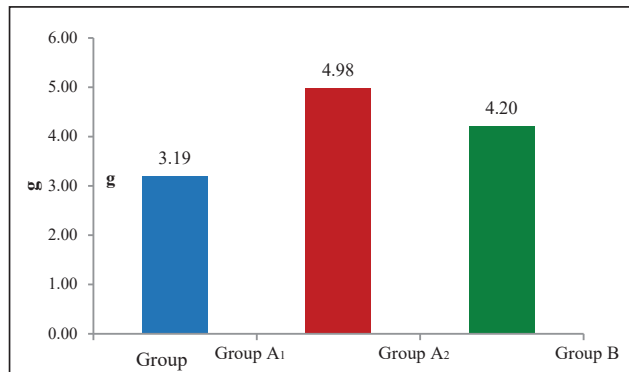
Group A1: Baseline control group

Group A2: Paracetamol treated control group

Group B: Experimental group (green tea pretreated and paracetamol treated group)

N = Total Number of rats

Figure 1: Mean initial and final body weight in different groups of rats (N=30)



Group A1: Baseline control group

Group A2: Paracetamol treated control group

Group B: Experimental group (green tea pretreated and paracetamol treated group)

N = Total Number of rats

Figure 2: Mean liver weight in different groups of rats (N=30)

The results are shown in table II and figure 3.

The mean (\pm SD) MDA level was 8.38 ± 1.94 , 18.14 ± 2.71 , and 11.38 ± 2.37 nmol/mg protein in group A₁, A₂ and B respectively.

The mean (\pm SD)MDA level was significantly higher in group A₂ and B ($p < 0.001$ and $p < 0.05$ respectively) in comparison to that of group A₁, whereas MDA level was significantly ($p < 0.001$) lower in group B than that of group A₂.

Table II: Malondialdehyde (MDA) levels in different groups of rats (N=30)

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Table II

MDA level in different groups of rats (N=30)

Group	MDA (malondialdehyde) (nmol/mg protein)
A ₁ (n=10)	8.38 ± 1.94 (5.56 - 11.31)
A ₂ (n=10)	18.14 ± 2.71 (14.67 - 22.08)
B (n=10)	11.38 ± 2.37 (8.86 - 15.98)

Multiple comparison

	MDA
	p value
A ₁ vs A ₂ vs B	0.000***
A ₁ vs A ₂	0.000***
A ₁ vs B	0.026*
A ₂ vs B	0.000***

Data are expressed as mean \pm 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.

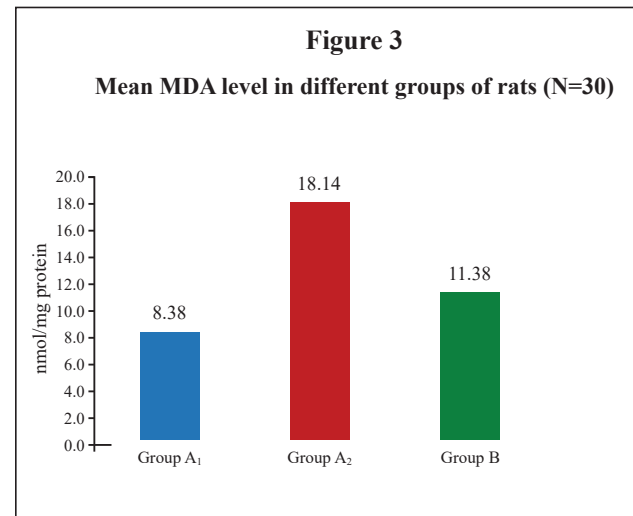
Group A₁: Baseline control group

Group A₂: 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

*=significant at p-value < 0.05; *** = significant at p-value< 0.001



Group A₁: Baseline control group

Group A₂: Paracetamol treated control group

Group B: Experimental group (green tea pretreated and paracetamol treated group)

N = Total Number of rats

Table III: Histological observation of liver in different groups of rats (N=30)

Group	Observation	Result/Findings
Group A ₁ (n=10) (Baseline control group)	<ul style="list-style-type: none"> • Architecture of hepatic lobule, central vein • Structure of hepatocyte, portal tract • Orientation of hepatic sinusoids 	Normal hepatic structure in all 10 rats
Group A ₂ (n=10) (Paracetamol treated control group)	<ul style="list-style-type: none"> • Presence of centrilobular necrosis • Disorganization of hepatic sinusoids • Infiltration of lymphocytes and Kupffer cells • Presence of fatty change • Ballooning degeneration 	Moderate histological changes in all 10 rats
Group B (n=10) (green tea pretreated and paracetamol treated group)	<ul style="list-style-type: none"> • Restoration of normal architecture of hepatic lobule and central vein • Normal structure of hepatocyte and portal tract • Less/absence of lymphocytic and Kupffer cells infiltration • Less/absence of centrilobular necrosis 	Normal histological findings in 7 rats and mild histological changes in 3 rats

Histological findings:

Normal:	Mild change:	Moderate change
Normal architecture of -hepatic lobule -central vein	-Less/absence of lymphocytic and Kupffer cells infiltration	Presence of centrilobular necrosis
Normal structure of -hepatocyte -portal tract	-Less/absence of centrilobular necrosis	Disorganization of hepatic sinusoids
Normal orientation of hepatic sinusoids		Infiltration of lymphocyte and Kupffer cells -Vacuolar degenerative changes in hepatocytes and pyknotic nucleus -Dilated blood vessels

The results are shown in table IV and figure 4.

In this study, histological examination of liver revealed normal findings in 100% of rats in group A₁. Whereas abnormal histological findings were observed in 100% of rats in group A₂. Again, 70% of rats in group B showed almost normal structure whereas 30% of them showed mild histological changes in liver.

Table IV: Distribution of rats by histopathological changes in liver (N=30)

Table IV
Histopathological findings of liver in different groups of rats (N=30)

Group	Histological finding		p value
	Normal	Abnormal	
A ₁ (n=10)	10 (100.0)	0 (0.0)	
A ₂ (n=10)	0 (0.0)	10 (100.0)	<0.001
B (n=10)	7 (70.0)	3 (30.0)	

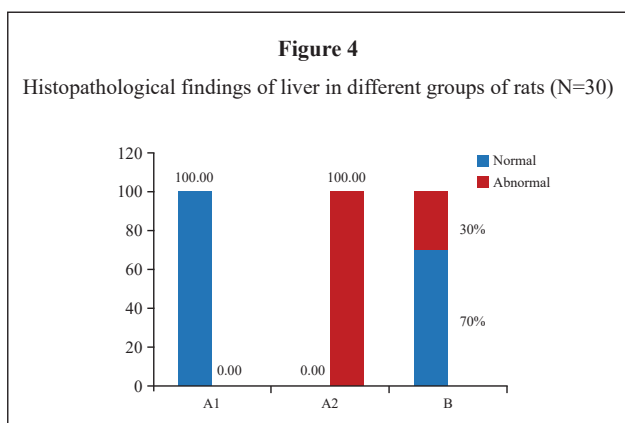
Statistical analysis was done by Chi-square test. Figures in parentheses indicate percentage.

Group A₁: Baseline control group

Group A₂: 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

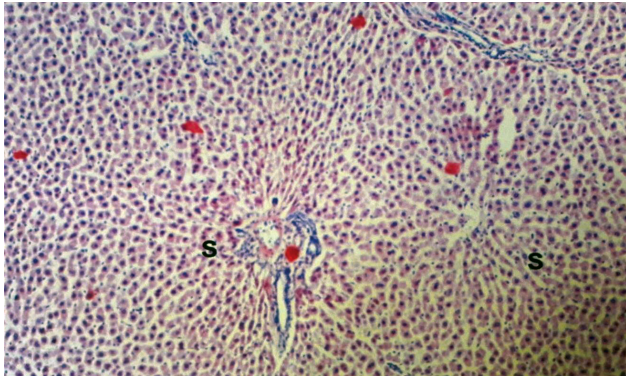


Group A₁: Baseline control group

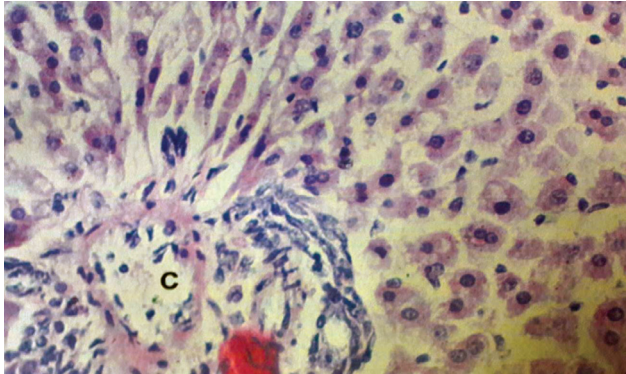
Group A₂: Paracetamol treated control group

Group B: Experimental group (green tea pretreated and paracetamol treated group)

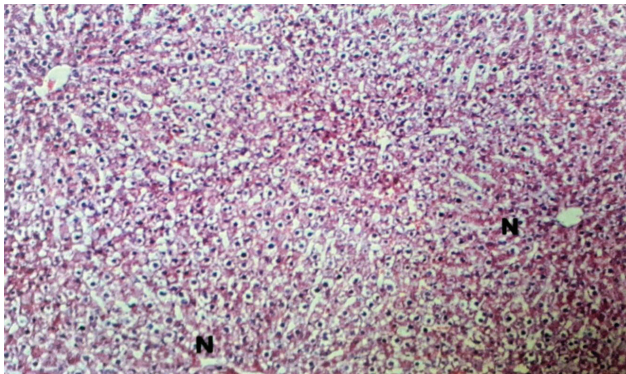
N = Total Number of rats



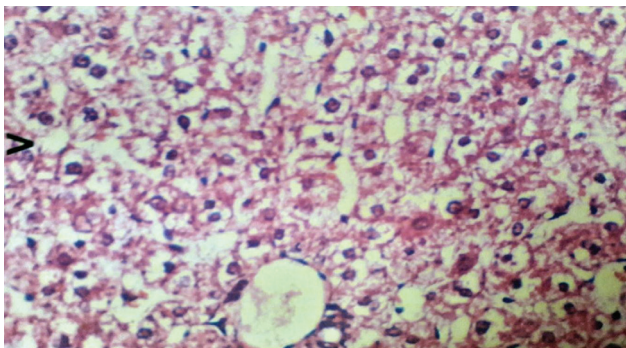
Photomicrograph 1: Architecture of liver of baseline control rats (here S represents hepatic sinusoid in X 100)



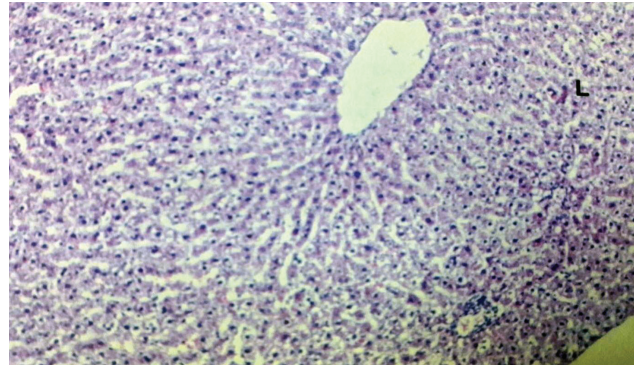
Photomicrograph 2: Architecture of liver of baseline control rats (here C represents central vein in X 400)



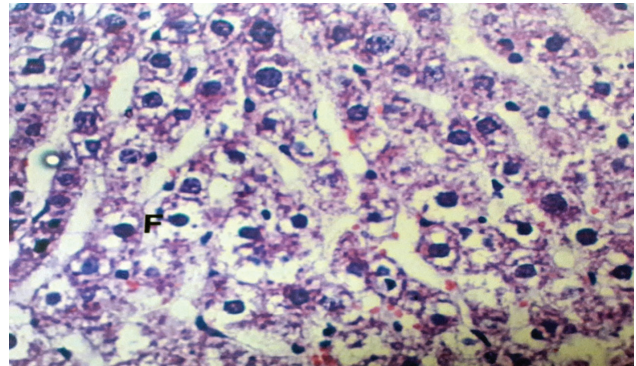
Photomicrograph 3: Liver of paracetamol treated control rats (here N represents centrilobular necrosis in X 100)



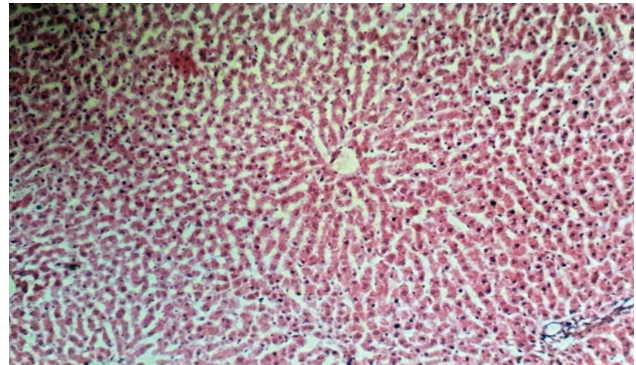
Photomicrograph 4: Liver of paracetamol treated control rats (here arrow mark > represents ballooning degeneration in X 400)



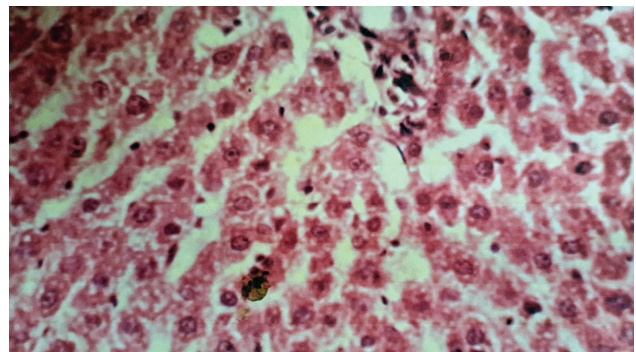
Photomicrograph 5: Liver of paracetamol treated control rats (here L represents lymphocyte infiltration in X 100)



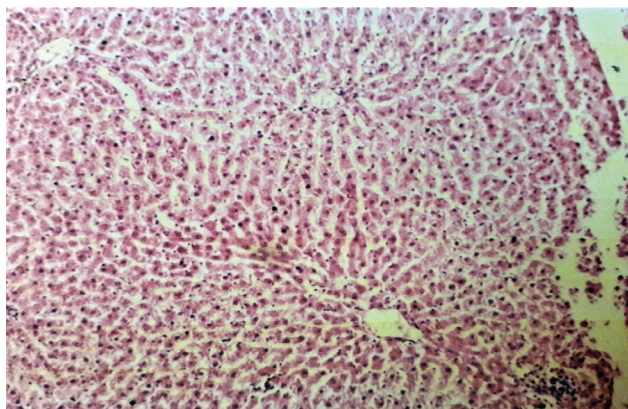
Photomicrograph 6: Liver of paracetamol treated control rats (here F represents fatty change of liver in X 400)



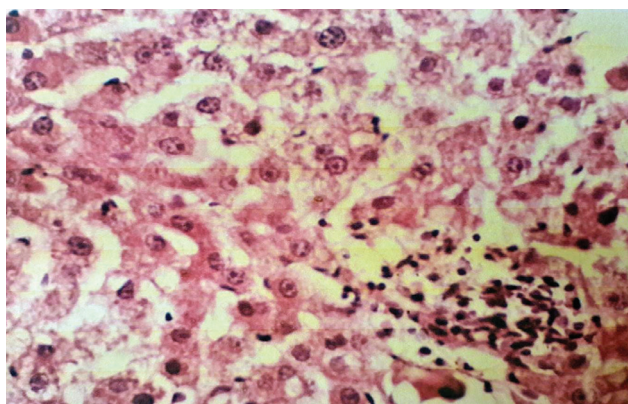
Photomicrograph 7: Improvement of necrosis and other changes of liver in green tea pre treated and paracetamol treated rats (X 100)



Photomicrograph 8: Improvement of necrosis and other changes of liver in green tea pre treated and paracetamol treated rats (X 400)



Photomicrograph 9: Improvement of necrosis and other changes of liver in green tea pre treated and paracetamol treated rats (X 100)



Photomicrograph 10: Improvement of necrosis and other changes of liver in green tea pre treated and paracetamol treated rats (X 400)

DISCUSSION:

In the present study, final body weight of baseline control group, paracetamol treated control group and green tea pretreated and paracetamol treated group was almost similar and the difference was not statistically significant. Almost similar finding was observed by other researchers²⁰. Whereas another researcher²¹ observed significant reduction ($p < 0.001$) of body weight after induction of hepatotoxicity by tamoxifen in comparison to that of baseline control group. The researcher also observed significant increase ($p < 0.001$) of body weight after green tea administration. The researcher suggested that this effect was may be due to toxic dose of tamoxifen and antioxidant effect of green tea.

In this study, liver weight was significantly ($p < 0.001$) higher in paracetamol treated control group and green tea pretreated and paracetamol treated group in comparison to that of baseline control group. Again liver weight was significantly ($p < 0.01$) lower in green tea pretreated and paracetamol treated group than that of paracetamol treated control group. Almost similar finding was observed by two other researchers^{22, 23}.

In this study, MDA concentration in liver tissue homogenate was significantly ($p < 0.001$) higher in paracetamol treated control group and green tea pretreated and paracetamol

treated group ($p < 0.001$ and $p < 0.05$ respectively) in comparison to that of baseline control group. Almost similar finding was observed by Deib and Ahmed²⁴, Mahboub²⁵, El-Aziz Tahoun et al.²⁶

Again MDA concentration in liver tissue homogenate 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 Elgawish et al.²⁷.

On the contrary, Lambert et al.²⁸ observed significant elevation of hepatic MDA level after 24 hours following administration of epigallocatechin-3-gallate (major tea polyphenol). The researcher suggested that this effect was may be due to induction of oxidative stress by epigallocatechin-3-gallate.

Moderate histological changes such as presence of centrilobular necrosis, disorganization of hepatic sinusoids, infiltration of lymphocytes and Kupffer cells, presence of fatty change and ballooning degeneration were observed in this study in paracetamol treated rats.

On the other hand, only minimal histological changes of liver were observed in 30% rats of green tea pretreated and paracetamol treated rats. These findings were also in agreement with those of different researchers of other countries Hasan and Ali¹⁵, Mahboub²⁵.

On the contrary, Zaki et al.²⁹ observed that high doses of green tea (500 mg/kg/day and 1000 mg/kg/day) had deleterious effect on liver histology resulting in congested central vein and hepatic sinusoids, hypertrophy of hepatic arteries, dilatation of bile ducts and cellular infiltration. The researcher suggested that this effect was may be due mitochondrial membrane collapse and formation of reactive oxygen species due to presence of epigallocatechin-3-gallate in green tea.

CONCLUSION:

From this study it may be concluded that green tea has hepatoprotective effect on paracetamol induced liver damage in Long Evans male rats.

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