EFFECT OF BETEL QUID ON CHEMICALLY INDUCED HEPATIC DAMAGE

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Summary

Research project deals with the screening of aqueous extract of betel quid with or without tobacco for hepatic damage activity. It has been reported that betel quid has parasympathetic activity and is used as vermicide and tamifuge in veterinary practice. Betel leaf was used for treatment of asthma and rheumatic arthritis in India. The animal models selected were carbon tetrachloride induced liver injury, paracetamol induced liver toxicity and thioacetamide induced liver necrosis in rats. Four different doses were selected betal quid without tobacco low dose (BQ LD), betal quid without tobacco high dose (BQ HD), betal quid with tobacco low dose (BQT LD) and betal quid with tobacco high dose (BQT HD) to carry out the research project. The functions were assessed by the estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum bilirubin, liver weight and histopathological studies. In the acute hepatitis models, the rats were pretreated with extracts and a single dose of hepatotoxin (carbon tetrachloride, paracetamol and thioacetamide) was administered and the results obtained were analyzed. The results of the present study are confusing hence further studies are required to conclude the effect of betel quid on chemically induced hepatic damage.

Keywords: Betel quid, Hepatic damage**,** Carbon tetrachloride, Paracetamol, Thioacetamide.

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Introduction

Liver disease is a worldwide problem. Liver is an organ of paramount importance as it plays an essential role in maintaining the biological equilibrium of vertebrates. The spectrum of function includes: metabolism and disposition of chemicals (xenobiotics) to which the organ is exposed directly or indirectly; metabolism of lipids, carbohydrates and protein; blood coagulation and immunomodulations[1]**.** The habit of chewing betel quid, containing fresh, dried or cured areca nut, catechu, slaked lime and flavouring ingredients wrapped in betel leaf is widespread in India, Pakistan, Bangladesh and Sri Lanka and in migrant populations from these regions in other countries[2]. Betel quid chewing is known to produce many pathological changes in the body. It is one of the leading causes for development of oral cancer and oropharyngeal tumor[3,4]. It can also lead to cholinergic crisis, cardiac arrhythmias, mild psychosis, milk-alkali syndrome, neurological problems, cardiovascular problems and gastrointestinal problems to name few of its effects[2].. Besides these, the tobacco and slacked lime present in betel quid can increase the plasma concentration of norepenephrine, epinephrine and also can induce hypercalcemia and metabolic alkalosis[5,6]. Furthermore, areca nut, a component of betel quid is shown to induce significant increases in the levels of cytochrome b5, cytochrome P-450, glutathione S-transferase and malondialdehyde (MDA) in dams and their pups[7]. The present work was undertaken to investigate the effect of betel quid on chemically induced hepatic damage with and without tobacco.

Methods:

Experimental animals - Wistar albino rats weighing 200-250 g of either sex were used. The experimental protocol was approved by the Institutional Animal Ethics Committee and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

***Preparation of betel quid extract and selection of dose*:**

a) Preparation of betel quid (with tobacco): Around 500 betel leaves were cut into small pieces and grinded with 3.5 liters distilled water to make a paste like mass. A powder of areca nut was made by crushing 500 gm of areca nuts (slice cutting) and powder of tobacco (312.5gm) tobacco was also made in the similar manner. Finally the betel leaf paste, areca nut, tobacco powder and 60gm of slaked lime were mixed well to make a paste and keep for 6hrs. After 6 hrs the paste was filtered and the filtrate was freeze-dried and stored –20 ºC till use.

b) Preparation of betel quid extract (without tobacco): The same procedure as mentioned above was followed except that tobacco was not added.

***Selection of dose and treatment period*:**
A treatment period of 10 days was followed to study effect in acute hepatic damage. The freeze dried extracts were mixed with the food and administered as follows:

Aqueous extract of Betel Quid without tobacco (BQ) low dose – 0.007% in food.
Aqueous extract of Betel Quid without tobacco (BQ) high dose – 0.014% in food.
Aqueous extract of Betel Quid with tobacco (BQT) low dose – 0.007% in food.
Aqueous extract of Betel Quid with tobacco (BQT) high dose – 0.015% in food.

***Evaluation of hepatic damage activity*:**

Acute hepatitis model:

i. Carbon tetrachloride induced acute hepatic injury: The CCl_4 was diluted with liquid paraffin (1:1) before administration. The animals were divided into 6 groups consisting of 6 animals for each. The animals were then subjected to either one of the following treatments for 9 days.

Group 1: Distilled water, (1ml/kg p.o.) for nine days.
Group 2: Distilled water for 9 days + CCl_4 (1ml/kg, p.o.) on ninth day.
Group 3: BQ low dose (0.007% in food) for 9 days + CCl_4 (1ml/kg, p.o.) on ninth day.
Group 4: BQ high dose (0.014% in food) for 9 days + CCl_4 (1ml/kg, p.o.) on ninth day.
Group 5: BQT low dose (0.007% in food) for 9 days + CCl_4 (1ml/kg, p.o.) on ninth day.
Group 6: BQT high dose (0.015% in food) for 9 days + CCl_4 (1ml/kg, p.o.) on ninth day.

Food was withdrawn 12hrs before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2, 3, 4, 5and 6. Single dose of CCl_4 (1ml/kg, p.o.) diluted with liquid paraffin (1:1) was administered on 9th day and sacrificed 24hrs after the administration of CCl_4. Blood samples were collected and the serum was used for assay of marker enzymes such as aspartate aminotransferase (AST)[8], alanine aminotransferase (ALT)[8], alkaline phosphatase (ALP). The liver was isolated and was washed with normal saline and blotted with filter paper and weighed. From one part of the liver, a homogenate was prepared for estimation of anti-oxidant enzymes; superoxide dismutase, catalase and glutathione and other part was used for histopathological studies[9]

ii. Paracetamol induced liver toxicity:
The same procedure as mentioned above was followed except that the liver was damaged using PCM (1g/kg, p.o.) diluted with sucrose solution (40% *w/v*). PCM was administered in 3 divided doses on day 9 and animals were sacrificed 48 hr after administration of PCM[10].

iii. Thioacetamide induced liver damage:
The same procedure was followed. Damage was induced by using TAA (100mg/kg, s.c), which was prepared in distilled water (2% solution)[11].

Statistical analysis
The significance of difference among the groups was assessed using one way analysis of variance (ANOVA) followed by Bonferroni's compare all pair of columns between the data of control and treated groups. The values expressed as mean $\pm$ SEM $p<0.05$ were considered significant.

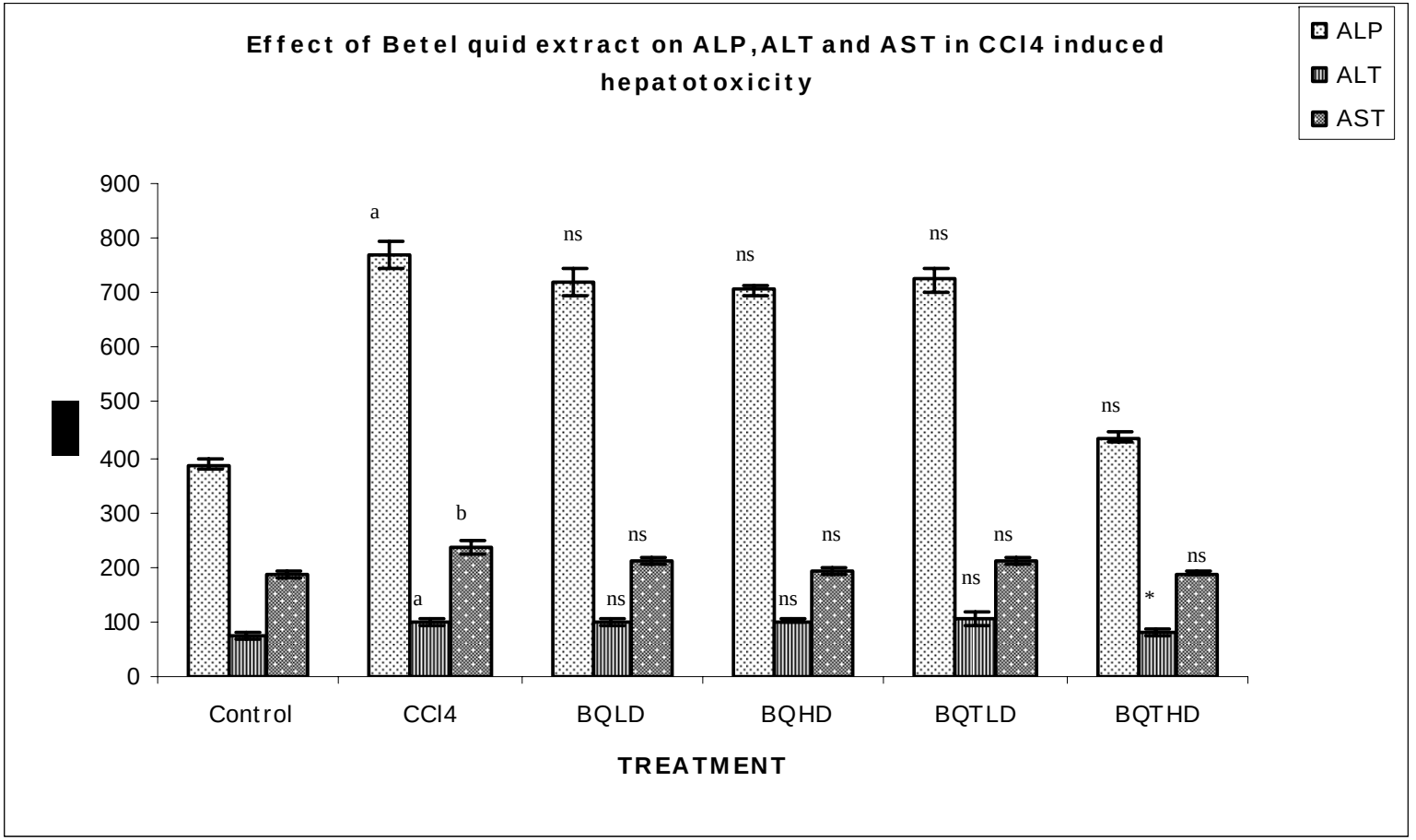
Results

Carbon tetrachloride induced acute toxicity - The parameters ALT, AST, ALP and bilirubin level in normal, control and treatment were analyzed. A significant difference in biochemical markers was observed between normal and CCl_4 control groups. Comparative analysis of the effect of various extracts on ALT, AST and ALP levels revealed that BQ LD (0.007% in food), BQ HD (0.014% in food), BQT LD (0.007% in food) and BQT HD (0.015% in food) was not significant when compared to control (Figure 1). BQT LD (0.007% in food) and BQT HD (0.015% in food) showed moderate significant effect on liver weight (Figure 2). Comparative analysis of the effect on serum bilirubin and between the extracts revealed that all the extracts did not show any activity (Figure 3). Histological examination of the liver tissue from CCl_4 treated animals revealed that CCl_4 had produced 5% necrosis on the liver cells (Figure 4a). BQ HD (0.014% in food) and BQT HD (0.015% in food) showed 15% (Figure 4b) and 70% (Figure 4c) necrosis on the liver cells respectively.

Paracetamol induced acute toxicity - The parameters ALT, AST, ALP and bilirubin level in normal, control and treatment were analyzed. A significant difference in biochemical markers was observed between normal and paracetamol control groups. Comparative analysis of the effect of various extracts on ALT, AST and ALP levels revealed that BQT HD (0.015% in food) showed moderate effect on ALT and AST whereas other did not show significant effect when compared to control (Figure 5). None of the extracts showed any effect on the liver weight (Figure 6) and bilirubin (Figure 7) when compared to control. Histological examination of the liver tissue from paracetamol treated animals revealed that paracetamol had produced 40% necrosis on the liver cells (Figure 8a). BQ HD (0.014% in food) showed 5% necrosis(Figure 8b) and BQT HD (0.015% in food) showed vaculation and around 10% necrosis, around 20% of cells showed early necrosis, cellular architecture deviates from normal architecture (Figure 8c).

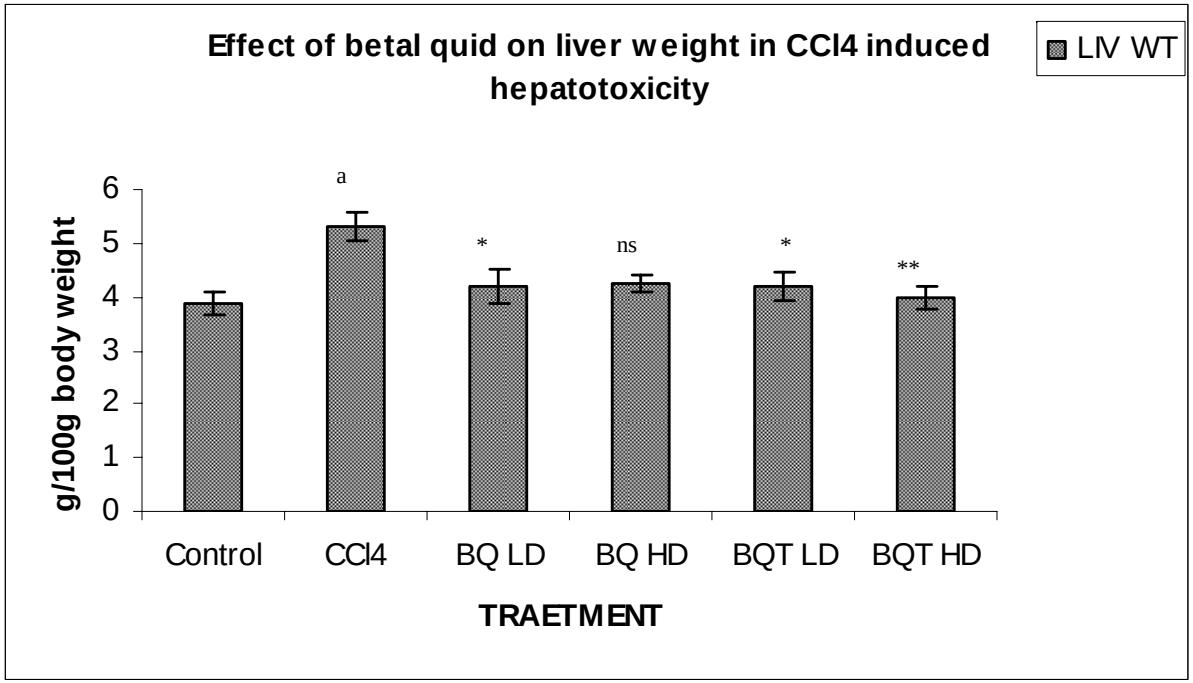
Thioacetamide induced acute toxicity - The parameters ALT, AST, ALP and bilirubin level in normal, control and treatment were analyzed. A significant difference in biochemical markers was observed between normal and thioacetamide control groups. Comparative analysis of the effect of various extracts on ALT, AST and ALP levels revealed that BQ LD (0.007% in food), BQ HD (0.014% in food), BQT LD (0.007% in food) and BQT HD (0.015% in food) moderately significant activity when compared to control (Fig 9). BQ HD (0.014% in food) and BQT HD (0.015% in food) showed significant effect on liver weight when compared to control whereas BQ LD (0.007% in food) and BQ HD (0.007% in food) did not show any significant effect on liver weight when compared to control (Figure 10). Comparative analysis of the effect on serum bilirubin and between the extracts revealed that none of the extracts showed any effect (Figure 11). Histological analysis of the liver tissue from thioacetamide treated animals revealed that thioacetamide had produced inflammatory reaction, infiltration of leucocytes and 30% necrosis on the liver cells (Figure 12a). BQ HD (0.014% in food) showed inflammatory reaction, infiltration of leucocytes and 5% necrosis on the liver cells (Figure 12b) whereas BQT HD (0.015% in food) showed inflammatory reaction, infiltration of leucocytes and 60% necrosis on the liver cells (Figure 12c).

Figure 1: Effect of Betel quid extracts on ALP, ALT and AST in carbon tetrachloride induced hepatotoxicity.



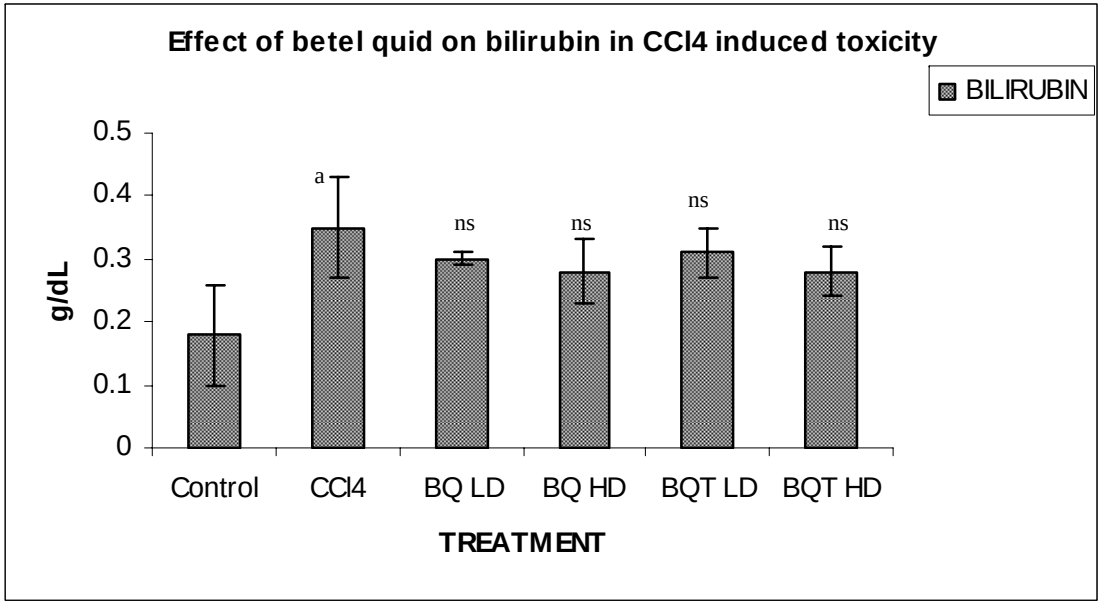
N = 6; Values are mean ± S.E.M, [a]$p<0.001$ vs. vehicle control, [b]$p<0.01$, vs. vehicle control, [ns]$p>0.05$, [*]$p<0.05$, vs. CCl_4 treated control.

Figure 2: Effect of Betel quid extracts on liver weight in carbon tetrachloride induced hepatotoxicity.



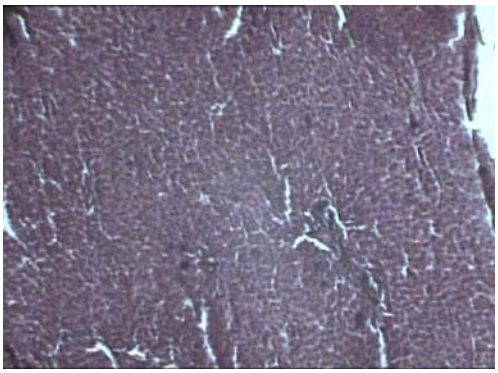
N = 6; Values are mean ± S.E.M, $^{b}p<0.01$, vs. vehicle control, $^{ns}p>0.05$, $^{*}p<0.05$, $^{**}p<0.01$, vs. CCl_4 treated control.

Figure 3: Effect of Betel quid extracts on bilirubin in carbon tetrachloride induced hepatotoxicity.



N = 6; Values are mean ± S.E.M, $^{a}p<0.05$ vs. vehicle control, $^{ns}p>0.05$ vs. CCl_4 treated control.

Figure 4: Effect of Betel quid leaf extract on CCl_4 induced acute liver injury (a: CCl_4 treated control, b: CCl_4 + BQ HD extract, c: CCl_4 + BQT HD extract. [H & E X100]).



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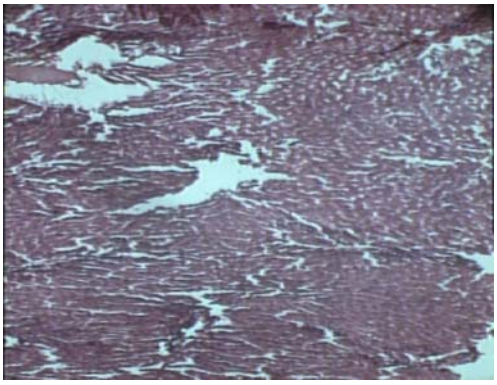

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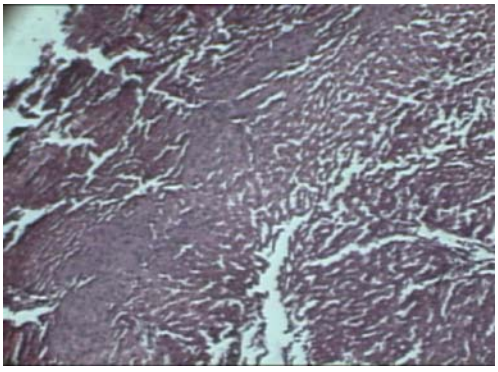

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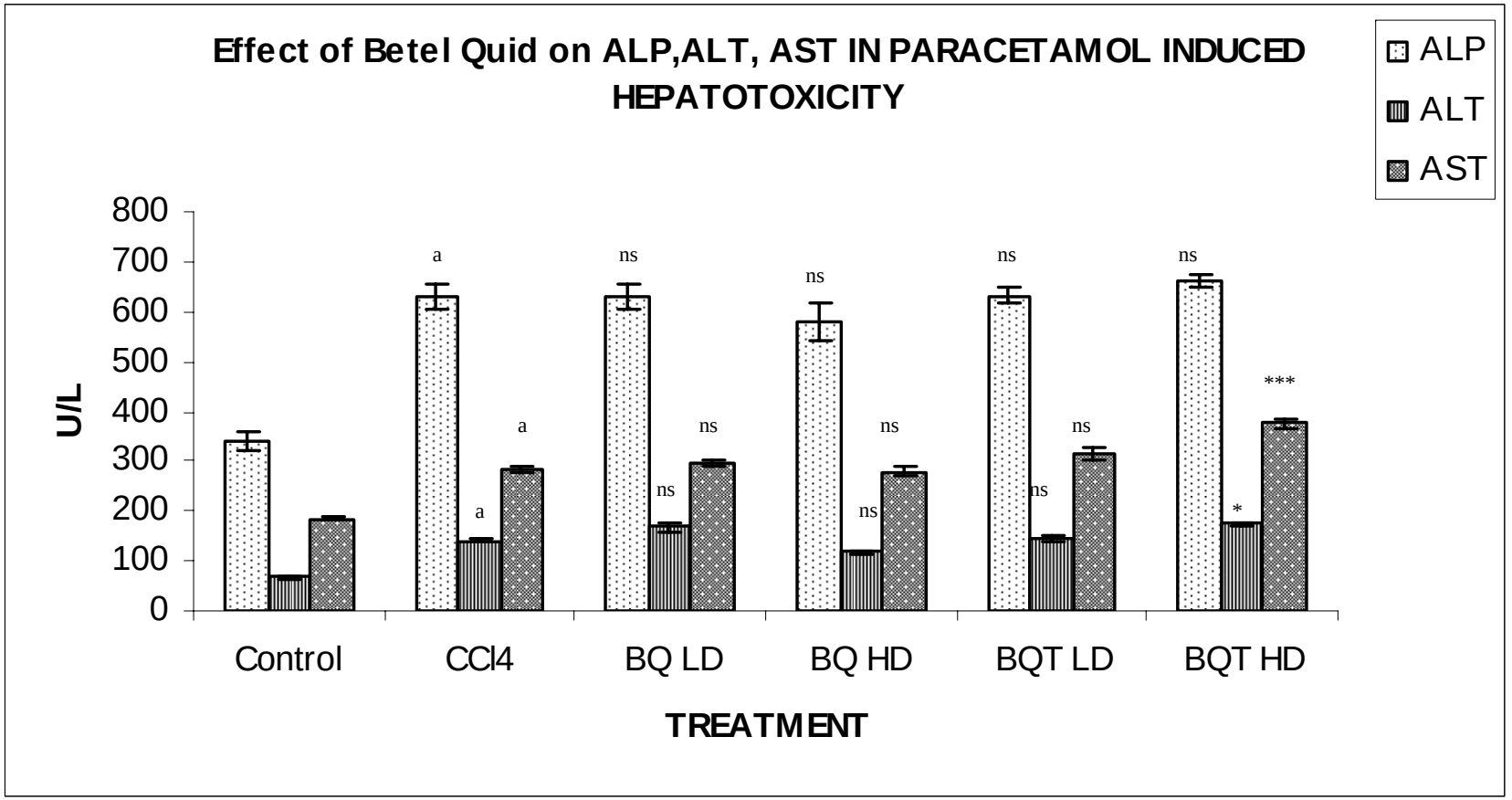
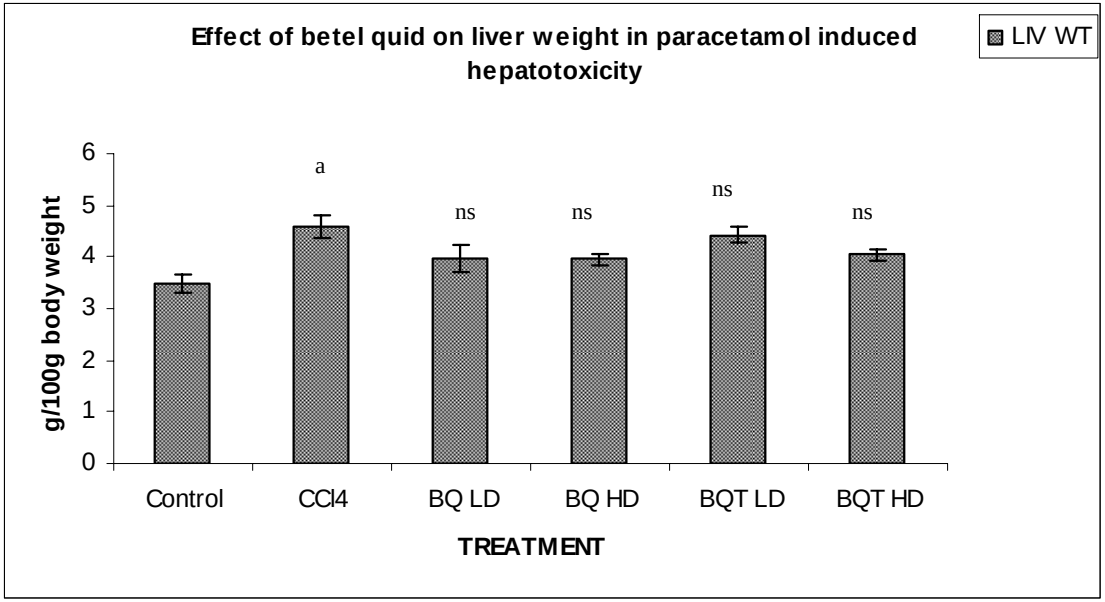
Figure 5: Effect of Betel quid extracts on ALP, ALT and AST in paracetamol induced hepatotoxicity.



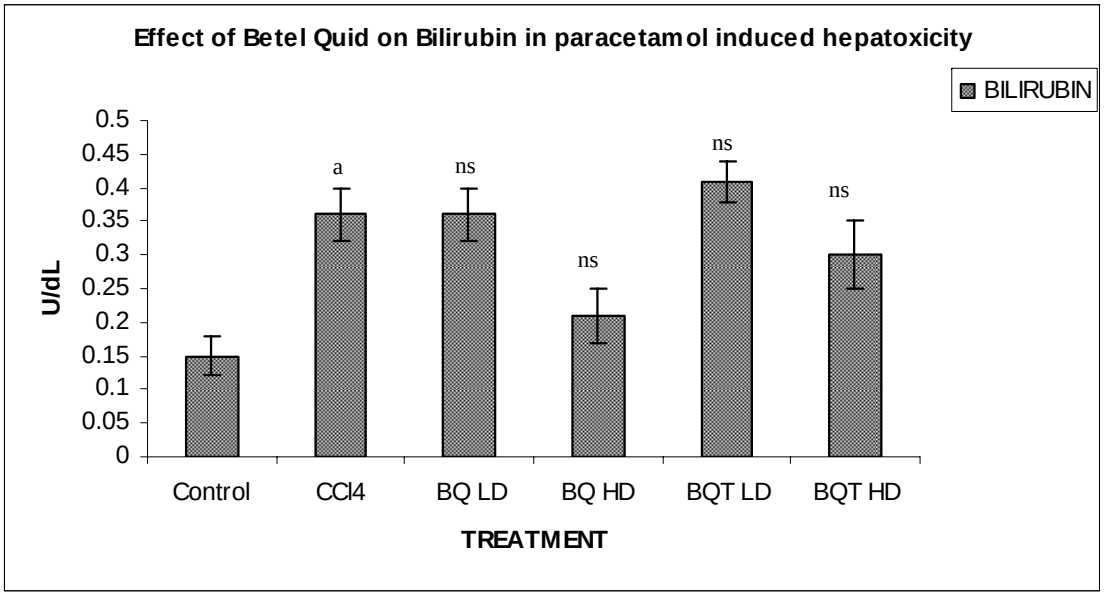
N = 6; Values are mean ± S.E.M, [a]p<0.001 vs. vehicle control, [ns]p>0.05, [*]p<0.01, [***]p<0.001 vs Paracetamol treated control.

Figure 6: Effect of Betel quid extracts on liver weight in paracetamol induced hepatotoxicity.



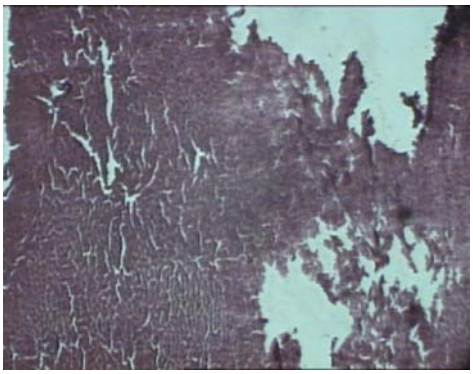
N = 6; Values are mean ± S.E.M, n = 6, [a]p<0.01vs. vehicle control, [ns]p>0.05 vs. Paracetamol treated control.

Figure 7: Effect of Betel quid extracts on bilirubin in paracetamol induced hepatotoxicity.



N = 6; Values are mean ± S.E.M, $^{a}p > 0.05$ vs. vehicle control, $^{ns}p > 0.05$ vs. Paracetamol treated control.

Figure 8: Effect of Betel quid leaf extract on Paracetamol induced acute liver injury (a: Paracetamol treated control, b: Paracetamol + BQ HD extract, c: Paracetamol + BQT HD extract. [H & E X100]).



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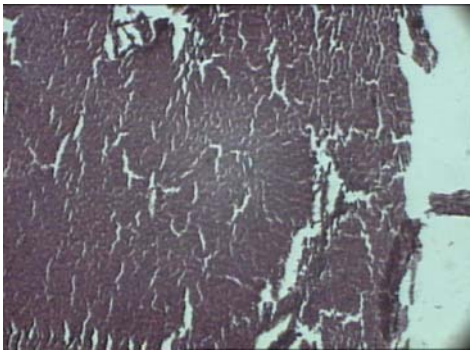

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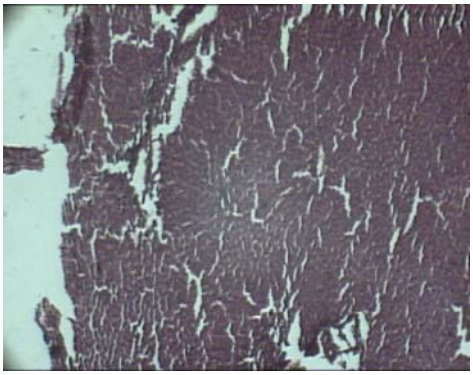
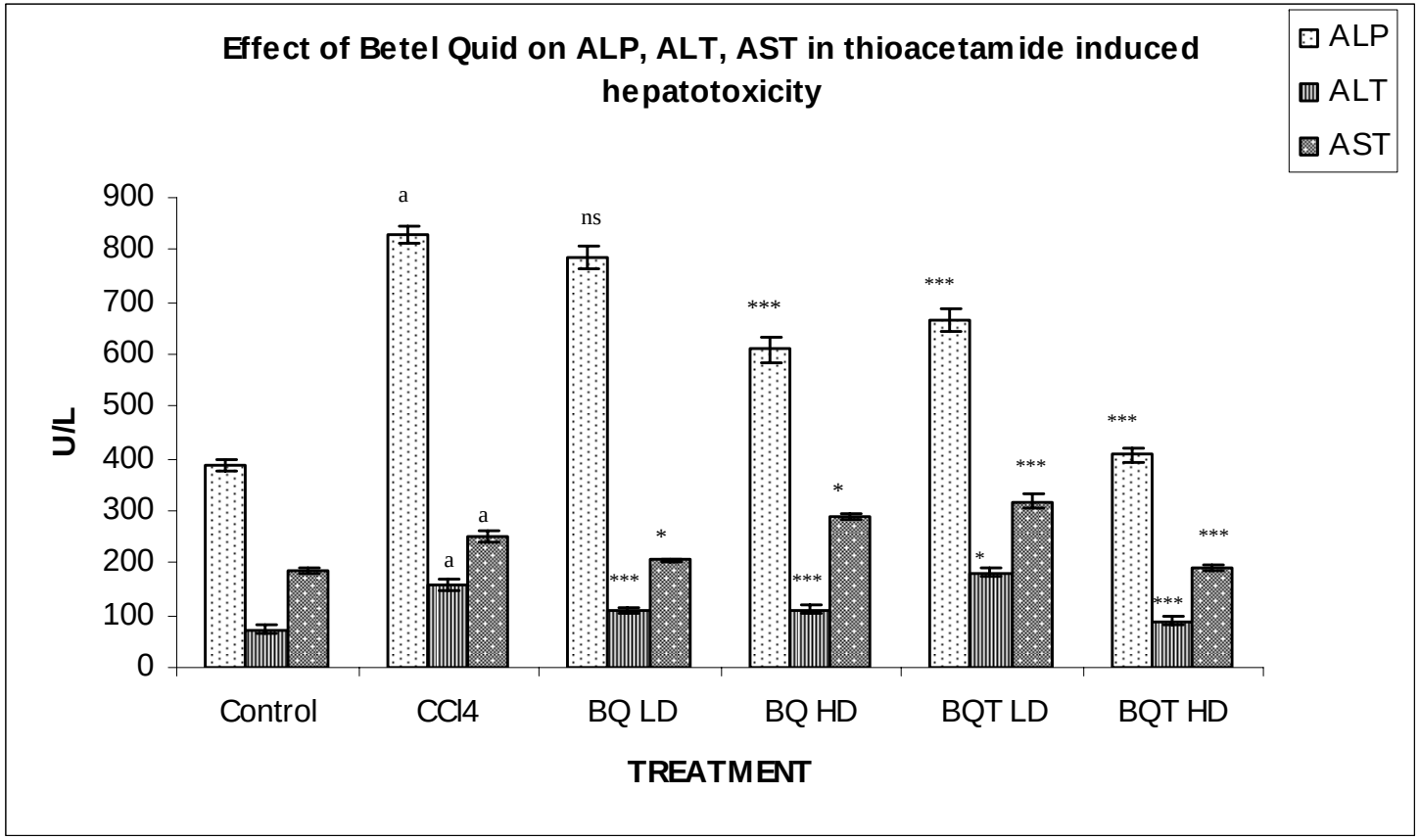


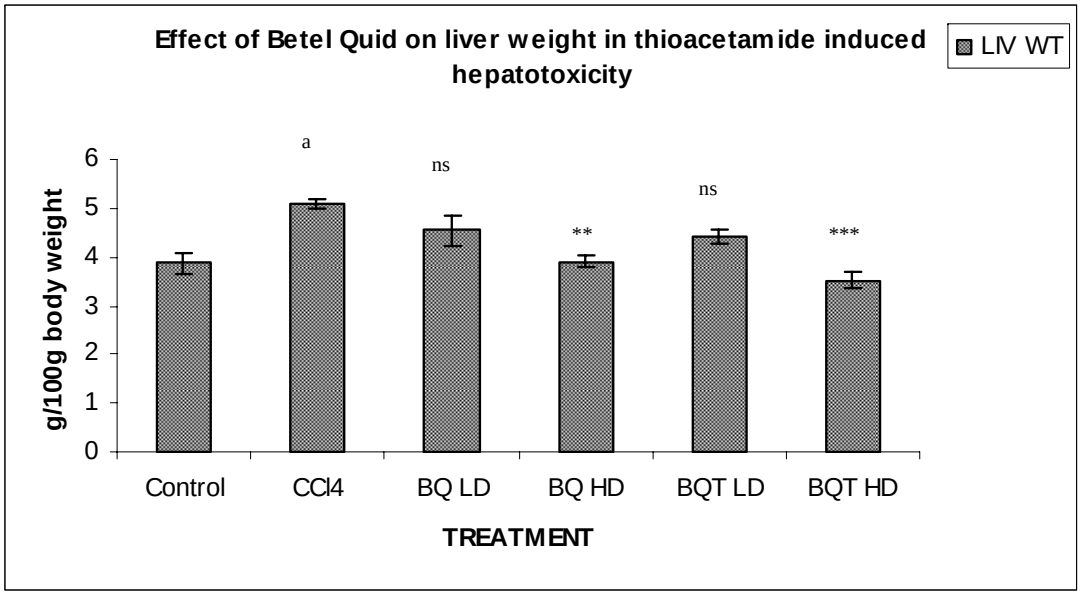
(c)

Figure 9: Effect of Betel quid extracts on ALP, ALT and AST in thioacetamide induced liver damage.



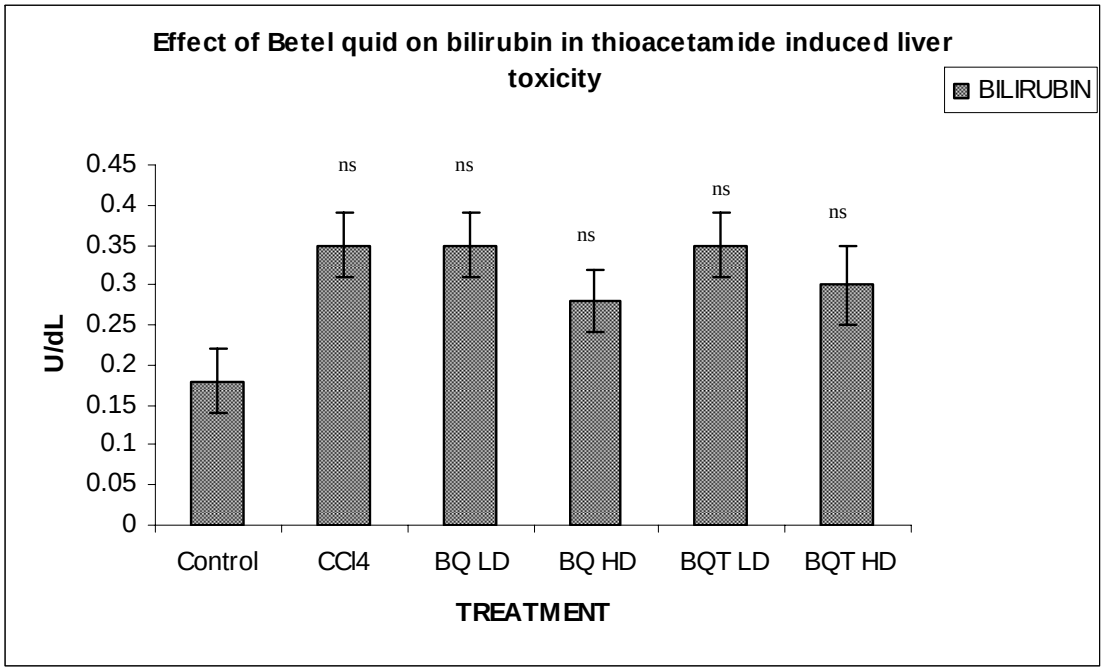
N = 6; Values are mean ± S.E.M, [a]p<0.001 vs. vehicle control vs. vehicle control, [ns]p>0.05, [*]p<0.05, [***]p<0.001 vs. Thioacetamide treated control.

Figure 10: Effect of Betel quid extracts on liver weight in thioacetamide induced liver damage.



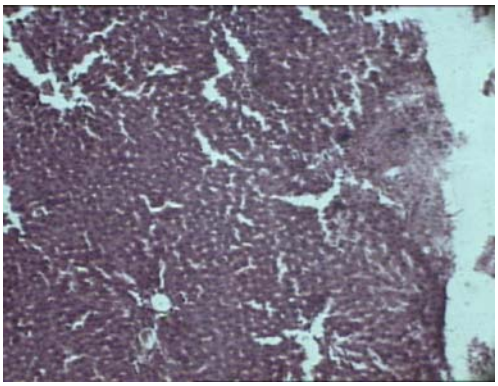
N = 6; Values are mean ± S.E.M, [a]p<0.01 vs. vehicle control, [ns]p>0.05, [**]p<0.01, [***]p<0.001 vs. Thioacetamide treated control.

Figure 11: Effect of Betel quid extracts on bilirubin in thioacetamide induced liver damage.



N = 6; Values are mean ± S.E.M, $^{ns}p>0.05$ vs. Thioacetamide treated control.

Figure 12: Effect of Betel quid leaf extract on Thioacetamide induced acute liver injury a: Thioacetamide treated control, b: Thioacetamide + BQ HD extract, c: Thioacetamide + BQT HD extract. [H & E X100]).



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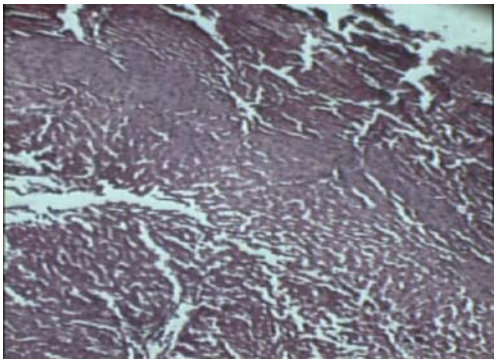

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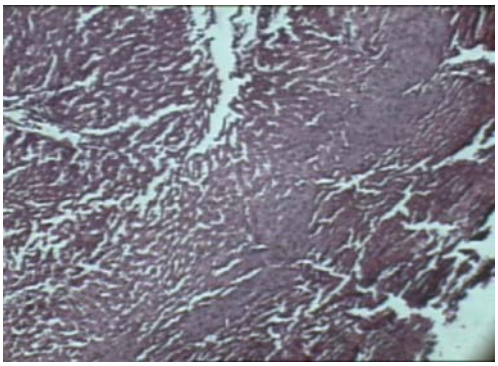

(c)

Discussion

The habit of chewing betel quid, containing fresh, dried or cured areca nut, catechu, slaked lime and flavouring ingredients wrapped in betel leaf is widespread in India, Pakistan, Bangladesh and Sri Lanka and in migrant populations from these regions in other countries[2]. Betel quid chewing is known to produce many pathological changes in the body. It is one of the leading causes for development of oral cancer and oropharyngeal tumor[3,4]. It can also lead to cholinergic crisis, cardiac arrhythmias, mild psychosis, milk-alkali syndrome, neurological problems, cardiovascular problems and gastrointestinal problems[2].

Arecanut, a component of betel quid is shown to induce significant increase in the levels of cytochrome b5, cytochrome P-450, glutathione S-transferase and malondialdehyde (MDA) in dams and their pups[7].

Although betel quid chewing is widely practiced in different parts of the world, its effect on hepatic damage has not been studied so far. The present study was undertaken to evaluate the effect of betel quid chewing on drug induced hepatic damage.

The mechanism by which CCl_4 manifests its injurious effects on the liver is varied. Since the liver is an organ with diverse functional activity, the hepatoprotective activity of a drug should be based on its ability to reduce the injurious effects or preserve the architecture and physiological functions of the liver, disturbed by a hepatotoxin[12].

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloro methyl radical. The activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum (E R) which is rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn give products like malondialdehyde that cause damage to the membrane. The lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl_4[13]. This is evidenced by an elevation in the serum marker enzymes namely ALT, AST, ALP and bilirubin. Drugs having antioxidant activity are effective in treating CCl_4 induced hepatotoxicity. BQ and BQT did not show significant result in CCl_4 induced hepatotoxic model. Normal histology of liver shows sinusoidal architecture of hepatocytes having no sign of necrosis or degeneration. Histopathological studies of acute model showed that CCl_4 caused 5% necrosis of the liver tissue. BQT high dose with CCl_4 caused 70% necrosis and BQ high dose with CCl_4 caused 15% necrosis.

It is known that paracetamol induces liver injury through the action of its toxic metabolite N-acetyl-p-benzoquinoneimine, produced by the action of cytochrome p-450. This metabolite reacts with reduced glutathione (GSH) to yield non-toxic 3-GS-yl-paracetamol. Depletion of GSH causes the remaining quinone to bind to cellular macromolecules leading to cell death[14].

Damages induced in the liver are accompanied by the increase in the activity of some serum enzymes. BQT and BQ did not show significant result in the serum enzymes in the liver of rats intoxicated with paracetamol following pretreatment with the extract. Histopathological studies showed that paracetamol had toxic effect on liver causing pyknosis, degeneration and severe congestion of the blood vessels with 40% necrosis.

BQT high dose with paracetamol showed 10% necrosis whereas BQ high dose with paracetamol showed very little vaculation and 5% necrosis.
Thioacetamide interferes with the movement of RNA from the nucleus to the cytoplasm, which may cause membrane injury. A metabolite of thioacetamide (S-oxide) is responsible for hepatic injury[14].
Pretreatment with BQ and BQT showed varying results in the serum enzyme markers treated with thioacetamide and most of them showed significant effect. Histopathological studies showed that thioacetamide caused perilobular hepatocyte necrosis, inflammatory reaction, infiltration of leukocytes with cytoplasmic vaculation and 30% necrosis. BQ high dose with thioacetamide showed inflammatory reaction as evident by leukocyte infiltration and around 5% necrosis. BQT with thioacetamide showed around 60% necrosis. The results obtained were varying and confusing and hence further studies are required on the betel quid combination with or without tobacco to conclude.

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