



Histopathological and Morphological Effects of Betel Quid with and Without Tobacco on the Liver of Adult Mice

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Abstract Objective: The main aim of this experimental research study is to explore the impact of Betel Quid (BQ) on the Histopathological and morphometric alterations in the liver of adult mice (Swiss albino mice), focusing on discerning any significant differences in Histomorphometric effects between the application of Betel Quid with and without tobacco. **Methods:** This study was conducted as a controlled experimental animal investigation at the Department of Anatomy and Postgraduate Laboratory of Isra University over a period of six months, involving the selection of 30 adult mice (Swiss albino mice) of varying genders. Inclusion criteria for the sample population consisted of healthy male mice weighing between 25-40 grams and healthy female mice weighing between 20-40 grams, aged between 3-6 months. Exclusion criteria included unwell mice, offspring, moribund specimens, pregnant mice, underweight and malnourished mice. Following a 30-day period of administering appropriate nutrition to each mouse, euthanasia was performed via cervical dislocation. Subsequently, the livers were extracted, rinsed with normal saline, assessed for gross morphological parameters (such as weight and size) and any Histopathologic macroscopic abnormalities were documented (such as Infiltration, necrosis, fatty changes and more). The livers were then fixed in 10% formalin for a duration of 48 to 60 hours. Histological observations were conducted on 4-micron-thick liver sections stained with Haematoxylin and Eosin using standard paraffin embedding techniques. The animals were categorized into three main groups, each consisting of 10 mice: 1: Control group A (n = 10) received a standard diet and clean water, 2: Experimental group B (n = 10) received a standard diet supplemented with BQ containing tobacco, 3: Experimental group C (n = 10) received a standard diet supplemented with BQ without tobacco. Each of these groups was further divided into two subgroups of 5 male and 5 female mice each for more detailed analysis. **Results:** The result revealed significant Histopathologic and morphometric effects in the livers of adult mice when comparing the control group A with the experimental group B who chewed Betel Quid with tobacco, group C who chewed Betel Quid alone without adding of tobacco, Both experimental groups induced statistically significant hepatic alterations compared to the control group (Group A, $p = 0.001$). Both treated cohorts exhibited marked reductions in overall body weight and liver weight (Group B 1.18 ± 0.13 g, Group C 1.33 ± 0.04 g, while the Control group A 1.63 ± 0.04 g, $p = 0.001$). Histopathological evaluation revealed extensive liver tissue damage across both experimental groups, characterized by significantly elevated rates of inflammatory infiltration, fatty changes, necrosis and collagen fiber fibrosis ($p = 0.001$ for all parameters versus control). Direct comparison between the experimental Groups B and C showed that tobacco co-administration resulted in a significantly greater loss in liver weight ($p = 0.004$), a similar outcome was observed in the comparison between control and experimental group B (mostly with p -value 1.000). Furthermore, no significant discrepancy was identified when comparing Histopathologic and morphometric effects between group C and group B in both genders ($p > 0.05$). In conclusion, it was determined that the consumption of BQ, with or without tobacco, induced similar significant effects on the livers of mice, notably affecting liver weight, degenerative alterations, fatty changes, necrotic manifestations, modifications in collagen fibers, liver infiltration changes and overall body weight. The comparative analysis between the two experimental groups led to the conclusion that the consumption of Betel Quid alone carries comparable risks to its consumption in conjunction with tobacco.

Key Words Histopathologic, Histomorphometric Effect, Betel Quid with and without Tobacco, Betel Quid, Liver of Mice

INTRODUCTION

Betel Quid (BQ) chewing is a longstanding traditional practice in the Indian subcontinent and various Asian regions, remaining prevalent today. BQ, often referred to as

"pan" by many, involves the chewing of a mixture typically containing Areca Nut, along with additional ingredients such as tobacco and betel leaf (piper betel). It is noteworthy that BQ ranks as the fourth most frequently used psychoactive

substance globally, following caffeine, alcohol and tobacco. Recently, the International Agency for Research on Cancer (IARC) has classified Betel Quid without tobacco as a human carcinogen [1-2].

The global consumption of BQ is estimated to range from 10-20% of the population, with approximately 600,000,000 individuals worldwide chewing Areca Nut. While consumers are dispersed worldwide, a significant concentration is observed in South and Southeast Asian countries, including China, Southeast Asian nations, Taiwan, Hainan Island, the Pacific Islands and regions with populations immigrated from South Asian countries like East and South Africa, the Malay Peninsula, North America and Europe.

In Pakistan, recent surveys conducted in low-income urban communities of Karachi have shown that 30-40% of adults consume *Areca catechu* in various forms such as BQ, gutka, *Areca catechu* and packaged chaalia, similar to Indian pan masala. Within Karachi, the Mohajir community, among other ethnic groups, exhibits a higher prevalence of *Areca catechu* product consumption. Adolescents tend to favor chaalia, whereas adults over the age of 30 prefer BQ.

In Bangladesh, a study on oral health screening in rural areas revealed that 40% of adults in Kishore Ganj utilized *Areca catechu* with tobacco and slaked lime in various combinations.

In Thailand, a study involving 4955 adults from rural areas aged 30-89 found that 17% of individuals were consumers of BQ. Since 1955, there has been a decline in BQ chewing in Thailand due to educational campaigns, although it remains more prevalent among the older population.

In rural Sri Lanka, chewing BQ is practiced widely. Urugoda and Stephen (1970) reported that 30.1% of 1088 individuals (27.9% males; 32.3% females) consumed BQ with tobacco. A large-scale survey in Sri Lanka showed that 57% of both males and females were the consumers.

In China, BQ chewing is common in southeastern regions, particularly in Hunan Province and Hainan Island, with an estimated 10% of the Chinese population of 2000,000 engaging in BQ consumption.

The overall prevalence of *Areca catechu* chewing was found to be 4.7%. The incidence was significantly higher in males compared to females, increased significantly with age and decreases notably with higher levels of education. Individuals residing in rural areas exhibited the highest incidence, followed by those in suburban and urban areas.

In Cambodia, a study involving 102 rural Cambodian women aged 39-80 disclosed that BQ chewing is predominantly popular among elderly women, while younger individuals seem less likely to adopt this practice.

In a recent study conducted in Malaysia across six different regions, it was found that out of 618 individuals surveyed, 114 were identified as Betel Quid (BQ) consumers. The consumption of BQ was notably higher among women, with 76.3% of consumers being female and 23.7% male. This pattern was particularly pronounced in

women over 35 years of age compared to those under 35. The practice of BQ consumption was most prevalent among individuals aged 15 to 20 years.

Migration of populations often introduces a variety of traditional practices to new societies. Research on chewing habits among migrant populations in Asia has revealed a prevalence of Areca Nut (AN) consumption either alone or in combination with Gutka and pan masala. These patterns closely mirror the chewing traditions prevalent in their countries of origin. Studies examining the incidence of areca nut chewing amongst migrant populations in the United Kingdom and South Africa have shown similarities to the prevalence observed in Indian populations migrating to the Malay Peninsula.

In the United Kingdom, "Asians in Britain" terms refers to individuals from Bangladesh, Pakistan, India, Sri Lanka and East Africa with roots in the Indian subcontinent. This demographic group constitutes approximately 3% of the total British population, with significant concentrations in areas like Greater and Inner London, West Midlands and West Yorkshire. Research on chewing habits among British Asians has primarily focused on these regions, where nearly half of the Asian population was born in the UK. Some studies have also examined the chewing habits of teenagers and young adults, indicating a shift towards older individuals in many countries while maintaining cultural significance in certain regions.

In various regions such as Thailand and Taiwan, fluctuations in the prevalence of chewing habits have been documented over time, with an increase in usage observed among younger populations. Population-based studies conducted in Pakistan, Nepal and India over the past few decades have indicated that 20-40% of individuals aged 15 and above are consumers of betel quid or *Areca catechu*. These studies have been carried out in specific regions within these countries, such as Maharashtra and Karnataka in India, Karachi in Pakistan and select areas in Nepal [3].

Various forms of BQ are available for consumption, including Paan masala, Gutka, Paan, mawa and khaini. Motivations for BQ consumption include pleasure, alleviation of fatigue, stimulation of saliva production, satisfaction and relief from toothaches. In India and neighboring countries, individuals may chew tobacco or dry BQ components alone or as a combination with Areca Nut, slaked lime and tobacco. Dry powdered blends containing lime, catechu, Areca Nut and various spices, with or without betel leaf and tobacco, are marketed in India. Notably, Pan masala is a tobacco-free product, while Gutka refers to products containing tobacco along with ingredients from Pan masala [4].

The primary constituents of Pan include Areca Nut (*Areca catechu*), betel leaf, Piper betel, slaked lime and catechu. Additional ingredients such as sweetening agents, spices and tobacco may be added based on individual preferences. The preparation involves placing these constituents on a betel leaf, which is then folded to form a triangular package, with or without tobacco. Catechu, a

reddish-brown component, is commonly derived from the heartwood of *Acacia catechu* trees found in Myanmar and India. *Areca catechu*, the key component of BQ, is a palm tree fruit grown in the Pacific Islands and Southern and Southeast Asia, known for its chemical composition including polyphenols, fiber, proteins, fats, carbohydrates, minerals and alkaloids like Guvacoline, guvacine, arecoline and arecaidine. Moreover, *Areca catechu* contains vanadium, manganese, sodium, magnesium, calcium and copper [5-8].

Betel leaf, a plant cultivated in South Asian countries and India, contains betel oil with phenolic combinations like eugenol, hydroxychavicol and chavicol, along with carotenes and vitamin C. Slaked lime, obtained from limestone or seashells, is a common ingredient in BQ and is prepared by roasting, grinding into a fine powder and mixing with water. The pH of slaked lime from limestone or seashells remains consistent. Additionally, tobacco used in conjunction with BQ is sourced from partially fermented and sun-dried leaves of *Nicotiana rustica* and *Nicotiana tabacum*.

Concerns raised by health experts regarding the increasing consumption of Areca Nut in South Asian nations, China and Taiwan have led to heightened surveillance efforts. In regions such as the Pacific Islands and South and Southeast Asia, the prevalent method of BQ consumption involves incorporating smokeless tobacco. While BQ is consumed without tobacco in New Guinea, Taiwan and China, a significant portion of consumers in these areas are also cigarette smokers. Migration has facilitated the spread of BQ habits from these regions to immigrant populations in other countries. Notably, in South Asian countries, commercially manufactured dry blends of Areca Nut and BQ ingredients (excluding betel leaf) are available in sachets, with Gutka being a popular form containing tobacco.

Studies from various countries have reported fluctuations in Areca Nut consumption trends, with declines noted in some Southeast Asian nations like Vietnam, Thailand, Indonesia and Cambodia, while increases have been observed in China, Taiwan and India. These trends are influenced by factors such as the introduction of Gutka and Areca Nut products like Pan masala.

In India, national surveys have estimated the popularity of Areca Nut chewing in males around 30 and 7% in females, with a significant proportion consuming tobacco. This survey reported that around 36.5% of males and 8.4% of females aged 15-49 years engaging in the consumption of various tobacco products such as gutka, pan masala and other forms of tobacco. With the absence of tobacco in pan masala were noted. Given that tobacco is commonly consumed in the form of Betel Quid (BQ) in many regions of India, often in conjunction with *Areca catechu*, the popularity of "tobacco chewing" is slightly higher than that of *Areca catechu* consumption. Local investigations have revealed that BQ consumption rates can be as high as 80% among both genders school staff in Mizoram, while in Sikkim, 44.8% of male school staff were found to consume gutka.

The explanation given for the use of tobacco products, including those containing areca nut (such as pan masala, mawa and gutka, i.e., BQ), among non-teaching college staff in Mumbai includes peer pressure, family influences. As well as the media influences from movies [9].

Pakistan faces significant health challenges related to Hepatitis C Virus (HCV) and associated liver diseases, with more deaths occurring due to hepatic diseases caused by HCV than terrorism in a year. The country carries a substantial burden of end-stage liver disease, hepatocellular carcinoma (HCC) and liver failure. Hepatitis is among the top ten notifiable diseases in Pakistan, with the country ranking second in HCV incidence globally after Egypt. The Sind province alone has approximately 1,000,000 chronic Hepatitis B Virus (HBV) carriers and 1,700,000 chronic HCV carriers. [10] Delta hepatitis (HDV) co-infection with HBV is prevalent in regions with high HBV incidence, with rates reaching 28.3% in Karachi and 60.7% in other parts of Sindh [11].

Cirrhosis is a primary cause of mortality in Pakistan, along with the liver cancer that is ranked as the sixth most common cancer worldwide. The incidence of HCC is higher in males (with the prevalence of 12 per 100,000) compared to females (4 per 100,000), typically occurring in individuals during their fourth and fifth decades of life. The majority of HCC cases (around 96%) develop in the post-cirrhotic stage among those chronically infected with HCV. Betel quid consumption has been associated not only with the development of oral cancer or oral precancerous conditions and HCC but also with hypertension, obesity, type 2 diabetes mellitus, cardiovascular disease, breast cancer and adverse pregnancy outcomes. Studies have shown a significant association between betel quid consumption and the risk of HCC and hepatic cirrhosis progression [12-13].

Objective of Study

The main aim of this experimental research study is to investigate the histopathological impacts, such as fatty alterations, infiltration, necrotic modifications and changes in collagen fibers, induced by the consumption of betel quid with and without tobacco on the livers of mature mice. Additionally, the study aims to assess the morphological consequences, including liver weight and liver size, resulting from the administration of betel quid with and without tobacco on the livers of the adult mice (Swiss albino mice). Furthermore, the research seeks to conduct a comparative analysis of the aforementioned Histopathologic and morphometric effects, if present, between the consumption of betel quid with and without tobacco on the livers of adult mice.

METHODS

The research was structured as an controlled experimental animal study, with the investigation taking place at the Department of Anatomy and the Postgraduate Laboratory of Isra University in Hyderabad, Sindh. The animal procedures were carried out at the Animal House Department of AHVS,

SU Tandojam. The study duration spanned six months. The sample consisted of 30 adult mice (Swiss albino mice) of diverse sexes, selected through a non-random sampling technique. Inclusion criteria encompassed male mice weighing between 25-40 grams, female mice weighing 20-40 grams and aged between 3-6 months. Exclusion criteria involved unwell mice, offspring, moribund subjects, pregnant individuals, underweight specimens and malnourished mice.

Animals and Protocol

The research conducted on animals followed the specific protocols that is approved by the Research Committee of Isra University Hyderabad (Approval #06893-19-6, on August 12, 2015) and adhered to the protocols outlined by the National Institutes of Health for the Use of the Laboratory Animals. Each animal was housed in plastic cages equipped with stainless steel food containers and plastic drinkers featuring stainless nozzles. Adequate water and standard chow diet were provided to the animals before and after the experiments. Bedding material such as sawdust was utilized and changed daily to ensure cleanliness. The animals were maintained in a well-ventilated and sanitary facility with a controlled room temperature of 26°C and a 12 hour light-dark cycle.

The control group (A) of animals received a regular diet along with unrestricted access to fresh water. In contrast, experimental group (B) animals were fed a diet consisting of 10% BQ and 10% Tobacco, supplemented with 80% normal chow diet, along with ad libitum access to clean water. Experimental group (C) animals were provided with a diet containing 10% BQ and 90% normal chow diet, accompanied by free access to water.

The animals were categorized into three main groups, each consisting of 10 animals:

- Control group A (n = 10) comprised of 5 male and 5 female animals receiving a normal chow diet and clean tap water
- Experimental group B (n = 10) included 5 male and 5 female animals fed a diet of normal chow mixed with BQ and tobacco in powder form
- Experimental group C (n = 10) consisted of 5 male and 5 female animals fed a diet of normal chow mixed with BQ without tobacco

Furthermore, each of the above groups was subdivided into two subgroups, with 5 male and 5 female animals in each subgroup:

- **Control group A1:** 5 male mice receiving a normal chow diet
- **Control group A2:** 5 female mice receiving a normal chow diet
- **Experimental group B1:** 5 male mice receiving a diet of normal chow mixed with BQ and tobacco

- **Experimental group B2:** 5 female mice receiving a diet of normal chow mixed with BQ and tobacco
- **Experimental group C1:** 5 male mice receiving a diet of normal chow mixed with BQ without tobacco
- **Experimental group C2:** 5 female mice receiving a diet of normal chow mixed with BQ without tobacco

Data Collection

Data gathering procedures involved the sacrifice of the animals after a 30 day period of receiving appropriate feed through cervical removal. The livers were extracted, rinsed with normal saline and subjected to assessment of gross morphological characteristics such as weight, size and any visible abnormalities. Subsequently, the livers were immersed in a 10% formalin solution for a duration of 48-60 hours. Histological slides were then prepared using standard paraffin embedding techniques, with 4-micron thick sections stained with Haematoxylin and Eosin for microscopic examination and analysis of tissue structures.

Data Analysis Procedure

The Data analysis conducted using SPSS version 21.0, with statistical significance determined at a p-value of less than 0.05. The comparison of variances between the control and experimental groups was performed through the application of the student t-test and Chi Square test.

RESULTS

The sample consisted of 30 mice, which were evenly distributed into three groups; 10 mice in experimental group B (BQ+tobacco), 10 in experimental group C (BQ without tobacco) and the remaining 10 in control group A.

Regarding morphological alterations, a decrease in liver weight was observed in 60.00% of the cases, affecting 18 out of the total 30 specimens. Similarly, minor variations in liver size were noted in 66.77% of the cases, specifically in 20 instances. No significant macroscopic changes in the liver were detected across any of the experimental groups or the control group. Furthermore, a notable reduction in body weight was identified in 66.77% of the cases, affecting 20 out of the 30 specimens.

Histologically, infiltrative modifications within the liver tissue were evident in 66.77% of the cases, specifically in 20 instances. Degenerative alterations were also prevalent, affecting 66.77% of the cases, with 20 specimens exhibiting such changes. Additionally, collagen fiber accumulation within the liver was observed in 66.77% of the cases, specifically in 20 instances. Fatty changes were present in 63.33% of the cases, affecting 19 specimens, while necrotic changes were identified in 63.33% of the cases, specifically in 19 instances. The findings are succinctly presented in Table 1.

The study's results highlighted significant Histopathologic and morphometric effects, particularly between the control group A and experimental group B, following the administration of Betel Quid with Tobacco on the livers of adult mice.

Table 1: Histologically, Infiltrative Modifications Within the Liver Tissue

Histomorphometric Effect		Frequency	Percent
Weight of liver	No change	8	26.67
	Increase	4	13.33
	Decrease	18	60.00
	Total	30	100
Size of liver	No changes	10	33.33
	Slight Changes	20	66.67
	Total	30	100
Gross changes of liver	No changes	30	100
	Yes	0	0.00
	Total	30	100
Infiltrative changes in liver	Yes	20	66.67
	No	10	33.33
	Total	30	100
Degenerative changes in liver	Yes	20	66.67
	No	10	33.33
	Total	30	100
Fatty changes in liver	Yes	19	63.33
	No	11	36.67
	Total	30	100
Necrotic changes in liver	Yes	19	63.33
	No	11	36.67
	Total	30	100
Change in collage fibers	Yes	20	66.67
	No	10	33.33
	Total	30	100
Change in body weight	no change	10	33.33
	decrease	20	66.67
	Total	30	100

Table 2: Comparison of Control Group A with Experimental Group B (n = 20)

Variables		Group		T-value	p-value
		Control Group A	Experimental group B (BQ+tobacco)		
Weight of liver (gm)	(Mean+SD)	1.63 +0.04	1.18±0.13	10.14	0.001
Size of liver (cm)	(Mean+SD)	1.95±0.52	2.0±0.56	-1.638	0.12
Gross changes of liver	No	10	10	1.000	
	Yes	0	0		
	Total	10	10		
Infiltrative changes in liver	Yes	0	10	0.001	
	No	10	0		
	Total	10	10		
Degenerative changes in liver	Yes	0	10	0.001	
	No	10	0		
	Total	10	10		
Fatty changes in liver	Yes	0	10	0.001	
	No	10	0		
	Total	10	10		
Necrotic changes in liver	Yes	0	10	0.001	
	No	10	0		
	Total	10	10		
Change in collage fibers	Yes	0	10	0.001	
	No	10	0		
	Total	10	10		
Change in body weight	decrease	0	10	0.001	
	No	10	0		
	Total	10	10		

A Comparative Analysis of Morphological and Histopathological Alterations Between a Control Group (A) and an Experimental Group (B)

Morphological Examination: The evaluation of liver weight, quantified in grams, exhibited a mean value of 1.63±0.04 in the control group (A) and 1.18±0.13 in the experimental group (B), demonstrating a statistically significant difference with a p-value of 0.001 (Table 2). Similarly, the assessment of liver size

in centimeters revealed a mean of 1.95±0.52 in the control group (A) and 2.0±0.56 in the experimental group (B), although this variance was not statistically significant ($p = 0.12$, Table 2). Analysis of gross changes in the liver indicated a non-significant difference between the groups, with a p-value of 1.000 (Table 2). Notably, a significant decrease in body weight was observed in the experimental group (B) compared to the control group (A), with a p-value of 0.001 (Table 2).

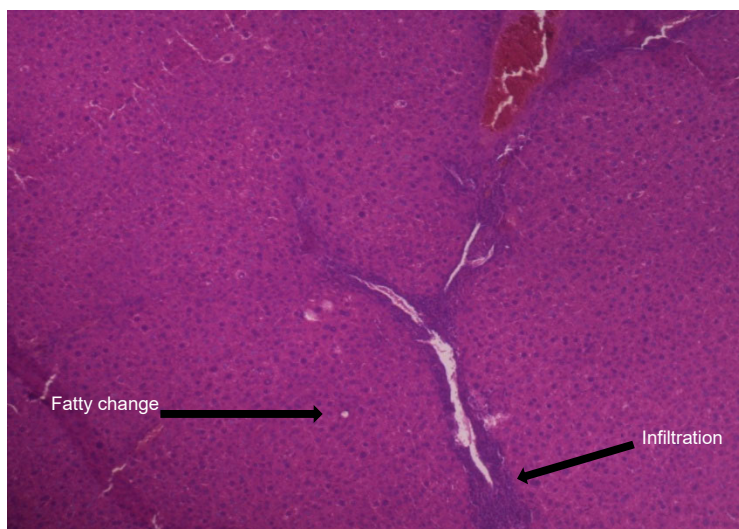


Figure 1: Microphotograph of the Cross-Section of the Liver, Showing Necrosis, Infiltration and Fatty Changes in Experimental Group B (H and E) stain $\times 100$

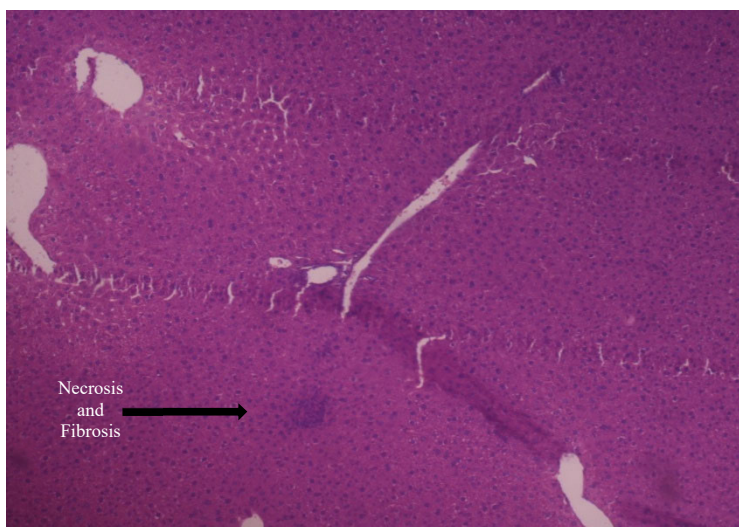


Figure 2: Microphotograph of the Cross-Section of the Liver, Showing Necrosis and Fibrotic Changes in Experimental Group B (H and E) stain $\times 100$

Histopathological Assessment

Regarding infiltrative changes, a significant increase was observed in the experimental group (B) compared to the control group (A), with a p-value of 0.001 (Table 2, Figure 1). Analysis of degenerative alterations revealed a notably higher significance in the experimental group (B) than in the control group (A), with a p-value of 0.001 (Table 2). Additionally, fatty changes were significantly more prevalent in the experimental group (B) than in the control group (A), with a p-value of 0.001 (Table 2, Figure 1). The incidence of necrotic changes in the liver was notably higher in the experimental group (B) than in the control group (A), with a p-value of 0.001 (Table 2, Figure 1). Moreover, the accumulation of collagen fibers in the liver was significantly more pronounced in the

experimental group (B) than in the control group (A), with a p-value of 0.001 (Table 2, Figure 1, 2). These histological findings are summarized in Table 2.

Conclusively, this study reveals a substantial histopathological and morphometric impact resulting from the exclusive administration of Betel Quid on the liver of adult mice, as evidenced by the significant morphological and histological disparities between the control group (A) and the experimental group (B).

A Comparative Analysis of Control Group (A) and Experimental Group (C)

One aspect under examination in this study concerns morphological alterations between the control group (A) and the experimental group (C). The liver weight, quantified in grams,

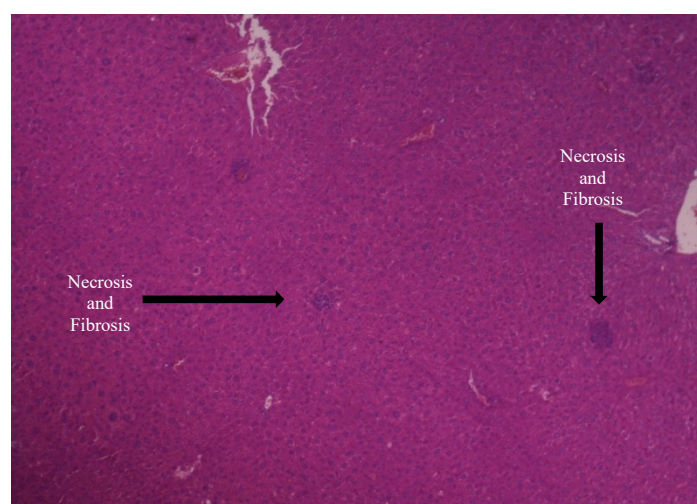


Figure 3: Microphotograph of the cross-section of the liver, showing necrosis and fibrosis changes in Experimental Group C (H and E) stain $\times 100$

Table 3: Comparison of Control Group A with Experimental Group C (n = 20)

Variables		Group		T-value	p-value
		Control group A	Experimental group C (BQ without tobacco)		
Weight of liver (gm)	(Mean+SD)	1.63 $\pm$ 0.04	1.33 $\pm$ 0.04	13.889	0.001
Size of liver (cm)	(Mean+SD)	1.95 $\pm$ 0.05	2.01 $\pm$ 0.05	-2.449	0.025
Gross changes of liver	No	10	10	1.000	
	Yes	0	0		
	Total	10	10		
Infiltrative changes in liver	Yes	0	10	0.001	
	No	10	0		
	Total	10	10		
Degenerative changes in liver	Yes	0	10	0.001	
	No	10	0		
	Total	10	10		
Fatty changes in liver	Yes	0	9	0.001	
	No	10	1		
	Total	10	10		
Necrotic changes in liver	Yes	0	9	0.001	
	No	10	1		
	Total	10	10		
Change in collage fibers	Yes	0	10	0.001	
	No	10	0		
	Total	10	10		
Change in body weight	decrease	0	10	0.001	
	No	10	0		
	Total	10	10		

displayed a mean of 1.63 ± 0.04 in the control group (A) and 1.33 ± 0.04 in the experimental group (C), with a statistically significant p-value of 0.001. This data is presented in Table 3. Regarding liver size, measured in centimeters, the mean size was 1.95 ± 0.05 in the control group (A) and 2.01 ± 0.05 in the experimental group (C), yielding a non-significant p-value of 0.25, as detailed in Table 3. Gross changes observed in the liver did not exhibit statistical significance when comparing the control group (A) and the experimental group (C), with a p-value of 1.000, as outlined in Table 3. Notably, a significant decrease in body weight was observed in the experimental group (C) compared to the control group (A), with a p-value of 0.001 (Table 3).

The histopathological alterations were also a focal point of the investigation. Significant infiltrative changes were

detected in the experimental group (C) compared to the control group (A), with a p-value of 0.001, which is visually represented in Table 3 and Figure 4. Moreover, the presence of degenerative changes in the liver was notably higher in the experimental group (C) in contrast to the control group (A), with a p-value of 0.001, detailed in Table 3. Fatty changes were significantly more prevalent in the experimental group (C) than in the control group (A), with a p-value of 0.001, with results presented in Table 3 and Figure 5. Additionally, necrotic changes were frequently observed in the livers of the experimental group (C) in comparison to the control group (A), with a p-value of 0.001 and these findings are displayed in Table 3 and Figure 3. Alterations in collagen fiber accumulation within the liver were also more commonly found in the experimental group (C) than in the

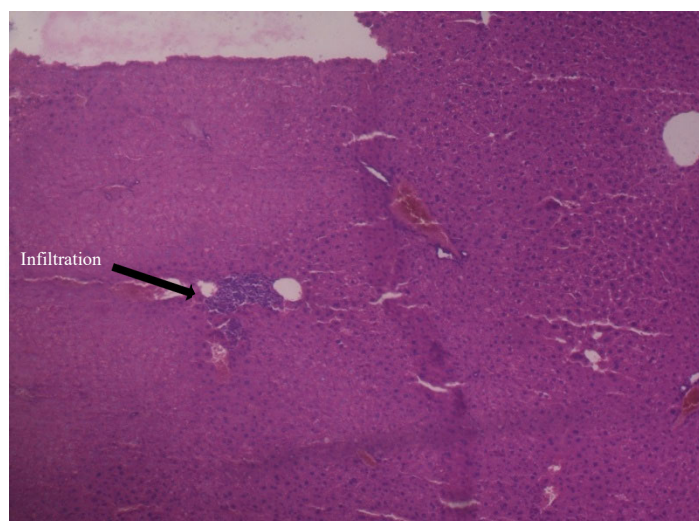


Figure 4: Microphotograph of the Cross-Section of the Liver, Showing Infiltrative Changes in Experimental Group C (H and E) stain $\times 100$

Table 4: Comparison of Experimental Group B with Experimental Group C (n = 20)

Variables		Groups		T-value	p-value
		Experimental group B (BQ+tobacco)	Experimental group C (BQ without tobacco)		
Weight of liver (gm)	(Mean+SD)	1.18 $\pm$ 0.13	1.33 $\pm$ 0.04	- 3.382	0.004
Size of liver (cm)	(Mean+SD)	1.99 $\pm$ 0.056	2.01 $\pm$ 0.056	- 0.788	0.44
Gross changes of liver	No	10	10	1.000	
	Yes	0	0		
	Total	10	10		
Infiltrative changes in liver	Yes	10	10	1.000	
	No	0	0		
	Total	10	10		
Degenerative changes in liver	Yes	10	9	0.500	
	No	0	1		
	Total	10	10		
Fatty changes in liver	Yes	10	9	0.500	
	No	0	1		
	Total	10	10		
Necrotic changes in liver	Yes	10	10	1.000	
	No	0	0		
	Total	10	10		
Change in collage fibers	Yes	10	10	1.000	
	No	0	0		
	Total	10	10		
Change in body weight	decrease	10	10	1.000	
	No	0	0		
	Total	10	10		

control group (A), with a p-value of 0.001, presented in Table 3 and Figure 3-5. The summary of these results can be found in Table 3.

The study revealed no significant histopathological and morphometric differences in the liver of mice exposed to Betel Quid with and without Tobacco. Notably, only a decrease in liver weight was observed in the experimental group (C) compared to the experimental group (B), while the remaining observations did not reach statistical significance levels.

Comparison Between Experimental Group B and Experimental Group C

The examination of morphological aspects revealed distinct differences between Experimental Group B and

Experimental Group C. The weight of the liver, measured in grams, exhibited a mean value of 1.18 $\pm$ 0.13 in Experimental Group B, whereas in Experimental Group C, it was notably higher at 1.33 $\pm$ 0.44 with a statistically significant p-value of 0.003 (Table 4). Furthermore, when considering the size of the liver in centimeters, Experimental Group B displayed a mean value of 1.99 $\pm$ 0.056, slightly lower than the mean of 2.01 $\pm$ 0.056 observed in Experimental Group C, although this variance was not statistically significant with a p-value of 0.44 (Table 4). Gross changes in the liver did not exhibit significant differences between Experimental Group B and Experimental Group C, as indicated by a p-value of 1.000 (Table 4). Additionally, reductions in body weight were comparable between the two groups, with no statistical significance observed (p-value 1.000, Table 4).

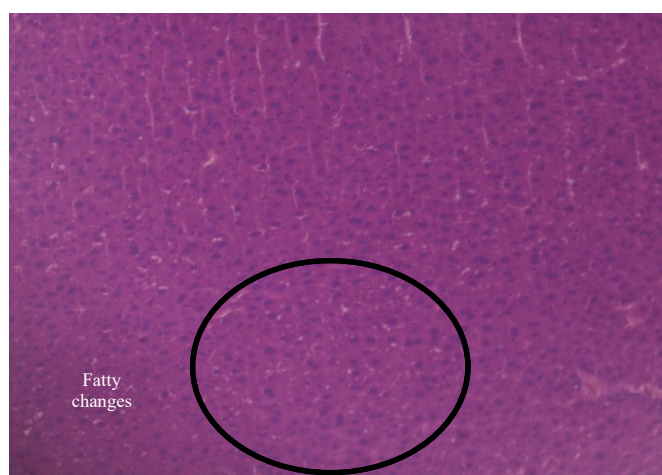


Figure 5: Microphotograph of the Cross-Section of the Liver, Showing Fatty Changes in Experimental Group C (H and E) stain $\times 100$

Histopathological analyses further elucidated the comparison between Experimental Group B and Experimental Group C. Infiltrative changes were found to be nonsignificant when contrasting Experimental Group B with Experimental Group C, with a p-value of 1.000 (Table 4). Similarly, degenerative changes in the liver did not significantly differ between the two experimental groups, as evidenced by a p-value of 0.500 (Table 4). Fatty changes and necrotic alterations also exhibited no significant distinctions between Experimental Group B and Experimental Group C, with p-values of 0.500 and 1.000, respectively (Table 4). Moreover, the accumulation of collagen fibers in the liver was found to be comparable between the two groups, with a p-value of 1.000 (Table 4).

The study outcomes summarized in Table 4 provide a comprehensive overview of the morphological and histopathological variances observed between Experimental Group B and Experimental Group C. Notably, the distribution of the 30 mice in the study was meticulously carried out, with equal gender representation ensured by assigning 5 males and 5 females to each of the three experimental groups.

In conclusion, the meticulous assessment of morphological and histopathological parameters in Experimental Group B compared to Experimental Group C elucidated notable differences in liver weight and subtle variations in liver size. While certain changes were statistically significant, others did not reach statistical significance, highlighting the complex interplay of factors influencing liver morphology and histopathology in experimental settings.

Gender disparities were evaluated between the control group (Group A) and the male and female subjects of the experimental group (Group B) in terms of morphological and histological alterations in the liver.

Regarding morphological changes, the liver weight was assessed in grams (gm), revealing a mean weight of 1.64 ± 0.05 in males and 1.62 ± 0.04 in females of Group A,

with a non-significant p-value of 0.54. In Group B, the male subjects exhibited a liver weight of 1.20 ± 0.15 , while females had a liver weight of 1.16 ± 0.11 , resulting in a p-value of 0.65. Similarly, liver size measured in centimeters (cm) displayed mean values of 1.96 ± 0.054 in males and 1.94 ± 0.54 in females of Group A, with a p-value of 0.58. In Group B, males had a liver size of 1.98 ± 0.044 , whereas females had a liver size of 2.00 ± 0.70 , yielding a p-value of 0.60. Gross changes in the liver showed no significant differences between genders of Group A and Group B, as indicated by a p-value of 1.000. Body weight reductions were consistent across genders in both Group A and Group B, with a p-value of 1.000.

Histopathological assessments revealed no significant differences in infiltrative changes between genders of Group A and Group B (p-value = 1.000). Similarly, degenerative changes in the liver exhibited non-significant variations in the comparison of genders between Group A and Group B (p-value = 1.000). Fatty changes were also insignificantly observed when comparing males and females of Group A with those of Group B (p-value = 1.000). Necrotic changes in the liver were similarly apparent in the gender comparison between Group A and Group B (p-value = 1.000). On the other hand, alterations in collagen fiber accumulation within the liver were comparable between genders of Group A and Group B, with a p-value of 0.292.

In summary, the findings from the morphological and histopathological analyses are synthesized in Table 5, illustrating the similarities and differences in liver characteristics between the control group and the experimental group across genders.

Gender differences were examined between the male and female subjects of the control group (referred to as Group A) and the male and female subjects of the experimental group (referred to as Group C) in terms of morphological and histological changes in the liver.

Regarding morphological changes, the weight of the liver, measured in grams, was analyzed. In the male subjects

Table 5: Gender Comparison of Control Group A with Experimental Group B (BQ+tobacco) (n = 20)

Variables		Genders		T-value	p-value
		Male	Female		
Weight of Liver (gm)					
Control A	(Mean+SD)	1.64±0.05	1.62±0.04	0.63	0.54
Experimental group B	(Mean+SD)	1.20±0.15	1.16±0.11	0.45	0.66
Size of liver (cm)					
Control A	(Mean+SD)	1.96±0.054	1.94±0.054	0.577	0.57
Experimental group B	(Mean+SD)	1.98±0.044	2.00±0.70	- 0.535	0.60
Gross Changes of Liver					
Control A	No	5	5	1.000	
	Yes	0	0		
Experimental group B	No	5	5		
	Yes	0	0		
Infiltrative Changes in Liver					
Control A	No	5	5	1.000	
	Yes	0	0		
Experimental group B	Yes	5	5		
	No	0	0		
Degenerative Changes in Liver					
Control A	No	5	5	1.000	
	Yes	0	0		
Experimental group B	Yes	5	5		
	No	0	0		
Fatty Changes in Liver					
Control A	No	5	5	1.000	
	Yes	0	0		
Experimental group B	Yes	5	5		
	No	0	0		
Necrotic Changes in Liver					
Control A	NO	5	5	1.000	
	YES	0	0		
Experimental group B	YES	5	5		
	NO	0	0		
Changes in Collagen Fibers					
Control A	No	5	5	0.292	
	Yes	0	0		
Experimental group B	Yes	5	4		
	No	0	1		
Change in Body Weight					
Control A	No change	5	5	1.000	
	Yes	0	0		
Experimental group B	Decrease	5	5		
	No	0	0		

of Group A, the mean weight was 1.64 grams with a standard deviation of 0.05, while in the female subjects of the same group, the mean weight was 1.62 grams with a standard deviation of 0.04, resulting in a p-value of 0.54. In contrast, in the male subjects of Group C, the mean liver weight was 1.34 grams with a standard deviation of 0.05 and in the female subjects, it was 1.32 grams with a standard deviation of 0.04, yielding a p-value of 0.54. The size of the liver, measured in centimeters, displayed differences between genders. In Group A, the mean liver size was 1.69 cm in males and 1.94 cm in females, with a p-value of 0.85. In Group C, the mean liver size was 2.02 cm in males and 2.00 cm in females, with a p-value of 0.60. Gross changes in the liver did not show significant differences between genders when comparing Group A and C, with a p-value of 1.000. Similarly, body weight decreases were consistent across genders in both Group A and Group C, with a p-value of 1.000.

Histopathological changes were also assessed. Infiltrative changes in the liver did not differ significantly

between genders in Group A compared to Group C, with a p-value of 1.000. Degenerative changes showed no significant variation in gender comparison between Group A and Group C, with a p-value of 1.000. Fatty changes were also not significantly different between males and females of Group A and Group C, with a p-value of 0.292. Similarly, necrotic changes did not exhibit significant differences in gender comparison between Group A and Group C, with a p-value of 0.292. Changes in collagen fiber accumulation in the liver did not significantly differ between genders in the comparison of Group A and Group C, with a p-value of 0.292. The summarized results of these findings are presented in Table 6.

In conclusion, the study revealed various morphological and histopathological changes in the livers of male and female subjects in both the control and experimental groups. While some differences in liver weight and size were observed between genders, gross changes, body weight decreases, infiltrative changes, degenerative changes, fatty changes, necrotic changes and collagen fiber accumulation

Table 6: Gender Comparison of Control Group A with Experimental Group C (BQ Without Tobacco) (n = 20)

Variables		Genders		T-value	p-value
		Male	Female		
Weight of Liver (gm)					
Control A	(Mean+SD)	1.64+0.05	1.62+0.04	0.63	0.54
Experimental group C	(Mean+SD)	1.34+0.05	1.32+0.04	0.15	0.88
Size of Liver (cm)					
Control A	(Mean+SD)	1.96+0.054	1.94+0.054	0.577	0.57
Experimental group C	(mean+SD)	2.02+0.044	2.00+0.070	0.535	0.60
Gross Changes of Liver					
Control A	No	5	5	1.000	
	Yes	0	0		
Experimental group C	No	5	5		
	Yes	0	0		
Infiltrative Changes in Liver					
Control A	No	5	5	1.000	
	Yes	0	0		
Experimental group C	Yes	5	5		
	No	0	0		
Degenerative Changes in Liver					
Control A	No	5	5	1.000	
	Yes	0	0		
Experimental group C	Yes	5	5		
	No	0	0		
Fatty Changes in Liver					
Control A	No	5	5	0.292	
	Yes	0	0		
Experimental group C	Yes	5	4		
	No	0	1		
Necrotic Changes in Liver					
Control A	NO	5	5	0.292	
	YES	0	0		
Experimental group C	YES	4	5		
	NO	1	0		
Changes in Collagen Fibers					
Control A	No	5	5	0.292	
	Yes	0	0		
Experimental group C	Yes	5	4		
	No	0	1		
Change in Body Weight					
Control A	No change	5	5	1.000	
	Yes	0	0		
Experimental group C	Decrease	5	5		
	No	0	0		

did not show significant discrepancies when comparing male and female subjects of the control and experimental groups. These findings contribute to the understanding of gender-specific responses in liver morphology and histology under experimental conditions.

The investigation delves into a gender-based comparative analysis of morphological and histopathological alterations within the experimental cohorts denoted as group (B) and group (C).

The assessment commenced with an evaluation of morphological attributes, starting with the weight of the liver. In the male demographic of group (B), the liver's weight was quantified at 1.22±0.15 gm, while in females, it amounted to 1.16±0.11 gm, yielding a non-significant p-value of 0.65. Conversely, in group (C), the male liver weight was 1.34±0.05 gm and in females, it was 1.32±0.04 gm, with a p-value of 0.54. A parallel examination of liver size revealed that in group (B), males exhibited dimensions of 1.98±0.044 cm, compared to 2.00±0.070 cm in females, resulting in a p-value of 0.60.

Similarly, in group (C), males displayed a size of 2.02±0.044 cm, while females manifested 2.00±0.070 cm, with a p-value of 0.60. Notably, gross hepatic changes exhibited a non-significant p-value of 1.000 when contrasting the genders of group (B) and group (C). Furthermore, body weight variations demonstrated comparable reductions in both male and female subjects within group (B) and group (C), indicating a p-value of 1.000.

The investigation extended to histopathological transformations, beginning with infiltrative alterations. In group (B), no significant variance in infiltrative changes was observed between genders, mirroring the findings in group (C), substantiated by a p-value of 1.000. Likewise, degenerative modifications within the liver exhibited non-significant distinctions in gender comparisons between group (B) and group (C), with a p-value of 1.000. Fatty changes, however, presented a non-significant disparity between males and females in group (B) versus group (C), with a p-value of 0.292. Similarly, necrotic adjustments were

insignificantly detected in the gender evaluation between group (B) and group (C), also yielding a p-value of 0.292.

Moreover, the investigation scrutinized collagen fiber variations within the hepatic tissue. The accumulation of collagen fibers depicted non-significant differences in gender comparisons between group (B) and group (C), aligning with the observations regarding fatty and necrotic changes, as evidenced by a p-value of 0.292. The comprehensive evaluation of morphological and histological parameters between male and female subjects within group (B) and group (C) accentuates the nuanced gender-specific responses within the experimental framework, elucidating the intricate interplay of biological factors influencing hepatic characteristics and functions.

DISCUSSION

The practice of Betel Quid chewing, a longstanding tradition across various regions in Asia, remains highly frequent today. In contemporary contexts, the term "Betel Quid" is commonly equated with "Pan" and its consumption in certain countries like Pakistan, India and neighboring regions. The term "quid" signifies a blend of constituents or substances that are placed in the mouth and subsequently ingested. In addition to Areca Nut, Betel Quid may consist of various ingredients and compositions, sometimes including tobacco, all encased in a betel leaf.

Although human epidemiological research indicate a correlation between betel quid consumption and liver disorders, there is a lack of controlled in vivo mouse experiments that systematically identify and evaluate the direct hepatic toxicity of betel quid with and without the inclusion of tobacco under unified dietary settings particularly in the Sindh province. International cohort studies, notably those conducted in Taiwan, have explored a strong correlation between Betel Quid use (excluding added tobacco) and liver conditions, particularly liver cancer.

The habit of Betel Quid consumption, whether with or without tobacco, has demonstrable impacts on the liver, including infiltrative changes, fatty alterations, modifications in collagen fiber accumulation, necrotic variations and alterations in liver weight.

The genetic and biological impacts of Betel Quid and its components, such as areca nut, manifest through a range of mechanisms. Prolonged local irritation of the oral mucosal cells induced by Betel Quid, particularly the constituents like areca nut and slaked lime, can instigate a state of persistent inflammation, elevated oxidative stress and heightened cytokine synthesis. The act of chewing Betel Quid results in the generation of reactive oxygen species and the formation of genotoxic substances, including arecoline and nitrosamines from areca nut, which have the potential to inflict DNA damage and genetic alterations in oral keratinocytes. The enduring oxidative stress imposed by these substances can propel the abnormal proliferation of cells and the development of hyperplastic or dysplastic lesions. The cumulative effect of these detrimental agents within the oral cavity of individuals habitually consuming Betel Quid may propel the progression

of pre-neoplastic cells towards malignancy. Conversely, a surge in reactive oxygen species can precipitate cellular structural impairment [14].

Noteworthy histopathologic and morphometric alterations were discerned in the livers of mice exposed solely to Betel Quid devoid of tobacco in this investigation. In this study the liver size displayed a statistically significant difference in both experimental groups. While there were no substantial gross alterations in the liver, distinctive degenerative and fatty changes were observed. Moreover, the presence of necrotic transformations, variations in collagen fibers, fluctuations in body weight and infiltrative modifications in the liver were markedly documented in association with the consumption of Betel Quid without the addition of tobacco, with notable p-values of 0.001.

In accordance with this experimental research outcomes, Wu *et al.* [15] conducted a population-based investigation which revealed a positive correlation between liver cirrhosis and hepatocellular carcinoma in individuals who specifically engaged in betel quid chewing. This study was carried out in Keelung, a region in northern Taiwan, involving the screening of 60,326 individuals aged 30 years and older who participated in a screening initiative. The study findings indicated notable exposure-response connections concerning the daily quantity of betel quids chewed, the duration of chewing, cumulative exposure (measured in portion-days) and the age at which the habit commenced, all exhibiting a positive association with the development of the disease ($p < 0.01$).

Furthermore, Wen *et al.* [16] conducted cohort studies in Taiwan to explore the relationship between the consumption of betel quid (without added tobacco) and the incidence of liver cancer. A case-control study and a cross-sectional study by Sun *et al.* [17] in Taiwan, alongside a case-control study from Thailand by Srivatanakul *et al.* [18] collectively demonstrated noteworthy correlations between the practice of betel quid chewing without added tobacco and the onset of hepatocellular carcinoma.

Betel leaf is recognized for its high safrole content, a product that classified as a carcinogen by reputable organizations such as the International Agency for Research on Cancer (IARC), the US Food and Drug Administration (FDA) and the European Commission for Health and Food Safety Authority (EFSA), leading to its prohibition for human consumption [19].

This research observed notable histopathologic and morphometric impacts on the livers of adult mice when exposed to Betel Quid combined with tobacco, yielding a significant. The observed effects included degenerative and necrotic alterations in liver tissue, changes in collagen fiber distribution, reduced liver weight and diminished body weight, all exhibiting a statistically significant impact with p-values of 0.001. However, alterations in liver size showed a non-significant change, while gross changes were also found to be non-significant.

Moreover, a study by Costiniuk *et al.* [20] conducted in Canada aligns with the outcomes of this research, indicating a correlation between tobacco exposure over time

(distinguishing between individuals who have ever smoked versus non-smokers and quantifying exposure in pack-years) and the progression towards significant liver fibrosis.

Zein *et al.* [21] and Yamini *et al.* [22] elucidated that the cumulative exposure to tobacco, as measured by pack years, exhibits a dose-dependent correlation with the severity of fibrosis in those diagnosed with non-alcoholic fatty liver disease, as evidenced by a study conducted in the United States. Additionally, a cohort study conducted by Tsochatzis *et al.* [23] in Greece, involving 217 participants, revealed a significant association between tobacco exposure and the progression of liver fibrosis.

Tobacco, whether consumed through smoking or in smokeless forms, encompasses various compounds including nicotine and phytochemical constituents such as the potent tobacco-specific carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 4-methyl-nitrosamino)-4-(3-pyridyl)-butanal (NNA) and N-nitrosornicotine, as well as heavy metals like Cadmium (Cd) and Mercury (Hg) and 23 polycyclic aromatic hydrocarbons. These components have been linked to the development of tobacco-related cancers and diseases [24-25].

Significant alterations in the liver weight and lipid content in mice were observed due to the consumption of Betel Quid combined with tobacco, with a statistically significant *p*-value of 0.001. Furthermore, a study by Kazi *et al.* [26] in Pakistan demonstrated fatty changes in the liver tissue of experimental mice offspring, with 14 out of 20 cases exhibiting fatty infiltration, of which 5 cases showed widespread hepatocyte swelling and ballooning indicative of hydropic degeneration.

Valenca *et al.* [27] conducted an animal study in Brazil, reporting that tobacco compounds induced fatty changes in the liver tissue, leading to alterations in the liver's histological structure, characterized by varying hepatocyte sizes with indistinct cellular boundaries, congested capillaries filled with erythrocytes and an increased presence of small fat globules indicating significant fatty degeneration in the liver due to oral nicotine consumption.

CONCLUSIONS

In our investigation, it was determined that the ingestion of Betel Quid, whether in conjunction with tobacco or independently, exerts notable impacts on the hepatic system of murine subjects. Noteworthy outcomes were particularly discerned concerning hepatic mass, as well as pertaining to lipid accumulation, necrotic manifestations, infiltrative alterations and the accrual of collagenous fibers within the hepatic tissue. Upon comparative analysis of the two distinct cohorts under experimentation, it was deduced that both experimental exposures were associated with hepatic histological alterations, while no statistically significant difference was detected between the two exposure groups for most assessed parameters.

To substantiate these assertions conclusively, it is imperative to undertake extensive animal studies employing ample sample sizes, which will facilitate the definitive establishment of these observations. Betel Quid utilization,

both singularly and in conjunction with tobacco blends and other variants, by systematically monitoring dose-dependent variations.

In order to ameliorate public health outcomes, enhance sanitary conditions, mitigate morbidity and diminish mortality rates, it is recommended that public health campaigns be orchestrated to disseminate information globally concerning the pernicious repercussions associated with Betel Quid consumption, with or without tobacco additives. Health regulatory bodies are advised to contemplate imposing restrictions on the commercial availability of Betel Quid, while concurrently advocating for the establishment of rehabilitation facilities tailored to individuals seeking cessation assistance.

Limitations

Limitations inherent in the present study encompass relatively small sample sizes, utilization of a murine model that might not entirely emulate human physiological processes and exposure parameters (including route, dosage and formulation) that do not precisely mimic the act of betel-quid chewing in humans. Evaluations were conducted at restricted time intervals predominantly employing semi-quantitative histological methods, lacking comprehensive biochemical or molecular elucidation of underlying mechanisms. Furthermore, the chemical composition of the betel quid mixtures was not thoroughly characterized, the potential effects specific to gender or age were not investigated and unidentified confounding factors (e.g., microbiome variations, cage-related influences) could have influenced the outcomes. Cumulatively, these constraints constrain the generalizability of the findings and underscore the necessity for broader, lengthier, dose-ranging, multimodal investigations, alongside supplementary epidemiological inquiries within human cohorts.

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