INTRODUCTION
Tobacco is the single most preventable cause of death in the world today. It is estimated that 5.4 million deaths currently occur every year due to tobacco use and projected figures show that by 2030 there will be more than 8 million deaths every year, of which 80% will take place in middle and low-income developing countries.

Tobacco is a plant that grows natively in North and South America. The main ingredient of tobacco compound is nicotine that is an alkaloid found in the nightshade family of plants Solanaceae which constitutes approximately 0.6–3.0% of dry weight of tobacco.

Use of smokeless tobacco (ST) is quite popular in countries of the Far East, Middle East and Europe. Chewing tobacco of different brands is available in most part of our country. The most common forms of smokeless tobacco (ST) available and used in Pakistan include: Pan/betel with tobacco – a chewed mixture of areca nut (Areca catechu), tobacco, catechu (Acacia catechu) and slaked lime (calcium oxide and calcium hydroxide), wrapped in a betel leaf (Piper betel) with sweetening agents. Naswar – a mixture of sun-dried, sometimes only partially cured, powdered local tobacco (Nicotiana rustica), ash, and oil, flavouring agents (e.g. cardamom, menthol), colouring agents (indigo) and lime. Chhaalia/supari – areca nut (used plain, flavoured with essences or coated with tobacco). Ghutka – sun-dried roasted, finely chopped tobacco, areca nut, slaked lime and catechu mixed with flavours and sweeteners.

Tobacco compounds have been observed to produce the fatty changes in the liver parenchyma and damage to the histoarchitecture of liver including the varied sizes of hepatocytes with uncertain cellular limits of histoarchitecture loss and the capillaries full

ABSTRACT
OBJECTIVE: To study the structural changes in the liver of the offsprings of mice exposed to smokeless tobacco during pregnancy.

MATERIAL & METHODS: Healthy female Swiss albino mice were mated. After confirmation of pregnancy 20 pregnant mice were divided into two groups, Group A was given Tobacco 5% mixed with normal diet and clean water ad libitum, while group B, the control was given normal diet with clean water ad libitum through out their pregnancy. After birth 20 offsprings (10 male & 10 female) were selected randomly from each group. 15 days after birth, the offsprings were sacrificed by cervical dislocation and their livers were dissected out for analysis.

RESULTS: The mean weight of liver in experimental male offsprings was found to be 0.58 ± 0.05 grams while in control group it was 0.82±0.03 grams. Where as the mean weight of liver in experimental female offsprings was found to be 0.50± 0.03 grams and in control group it was 0.78±0.02 grams. The difference in the mean weight of the liver between the experimental and control group was found to be highly significant (p value < 0.001) in both sexes. Fatty change was observed in the liver parenchyma of the experimental group in which 14 out of 20 cases of livers from the offsprings of experimental mice showed fatty infiltration out of which 5 cases were accompanied by wide spread swellings and ballooning of hepatocytes indicating hydropic degeneration.

CONCLUSION: The use of smokeless tobacco during pregnancy has some adverse effects on the offsprings manifested by the decrease in the weight of the liver of offsprings as well as cellular injury to liver parenchyma especially the fatty infiltration and hydropic degeneration.

KEY WORDS: Smokeless Tobacco, Liver, Offsprings, Mice.

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Tobacco compounds have been observed to produce the fatty changes in the liver parenchyma and damage to the histoarchitecture of liver including the varied sizes of hepatocytes with uncertain cellular limits of histoarchitecture loss and the capillaries full

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ORIGINAL ARTICLE
EFFECTS OF SMOKELESS TOBACCO ON THE DEVELOPING LIVER OF THE MICE
Afroz S. Kazi, Faisal Mughal, Mansoor Ali Khan, Zaheer A. Memon
with red blood cells. Further an increase in the number of fat globules of small size point that there is massive fatty change in liver due to oral use of nicotine.

Some studies have been conducted to ascertain the effect of nicotine on fetal growth and whether this could be related to the actions of this drug on maternal adipose tissue metabolism. It has however been speculated that nicotine which is present in tobacco smoke may cause uterine vasoconstriction, reduce maternal appetite, or in some way produce metabolic changes in the mother and/or fetus. The placenta plays a vital role in prenatal development by transporting nutrients and wastes between the maternal and fetal circulation and by providing hormones needed for normal development. Human placental explants can be grown and studied experimentally in vitro. In one such study, it was shown that nicotine alone was able to inhibit differentiation and thereby retard cytotrophoblast invasion in an in vitro assay. These authors further showed that nicotine inhibited synthesis and activation of type IV collagenase, which is necessary for cytotrophoblast invasion. Some placental studies have involved an interesting correlation of in vivo and in vitro experimentation. These data demonstrate that nicotine is a key molecule in tobacco that inhibits the growth and differentiation of cytotrophoblasts in the human placenta. Nicotine may lead to vasoconstriction and reduced blood flow. Nicotine can also increase maternal blood pressure and heart rate, reducing uterine blood flow.

The nicotine treated embryos also show altered development of the neuroepithelium, potentially contributing to the elevated rate of neural tube defects seen with. It has been postulated that fetuses adapt to environmental influences by changing their physiology, and in doing so, they irreversibly alter the structure and function of specific tissues in the body. The changes are likely beneficial/protective in the short-term, but they may be maladaptive in the long run. Some of these changes include augmented insulin resistance, modified reactivity of the hypothalamus- pituitary-adrenal axis, and reduction in the size/function of the kidneys, pancreas, and liver. Nicotine suppresses the mother’s appetite, leading to poor nutrition of the mother and fetus. Nicotine causes alterations in the cellular growth and activity of the central and peripheral nervous systems.

Nicotine in smokeless tobacco plays a significant role in fetal weight reduction, fetal death, dosage dependant hemorrhages, and a delay in ossification of the bones of the fetus. Smokeless tobacco use is associated with reduction of gestational age.

Histological changes have also been reported in the liver, since it is the major organ where nicotine, a component of tobacco is metabolised. Such disorders indicate that the increased use of smokeless tobacco is a cause of great burden on a developing nation like Pakistan. Although few studies are available on the effects of smokeless tobacco or nicotine on the liver, but none was observed on the effects of the local brand of tobacco on the developing liver in this region. The objective of the present research study was to observe the effects of the use of local chewing tobacco on the developing liver of the offsprings of mice.

**MATERIAL AND METHODS**

Healthy Swiss albino mice were obtained from the animal house of the Department of Animal Husbandry and Veterinary Sciences Sindh Agriculture University, Tandojam. Mice were housed in pairs (females) or individually (males) in polycarbonate cages (with saw dust bedding) in temperature-controlled (23°–26°C) and humidity-controlled (55% RH) rooms. The cages were equipped with stainless steel feed containers and plastic drinkers with stainless nozzles. Saw dust was used as beddings and was changed weekly. The animals were housed under a hygienic and well ventilated environment. Mice were provided food (lab chow) and tap water ad libitum. The light/dark cycle was maintained on 12 h intervals. All animal procedures were conducted under an animal protocol approved by Sindh Agriculture University, Tandojam.

All female mice were mated with adult male mice, after restraining from sex for seven days to increase their sexual urge for the opposite sex. Pregnancy was confirmed by presence of mucus vaginal plug between 1- 10 days of pairing. On confirmation of pregnancy the males were removed.

Mice were provided with the diet, of Lab.Cakes to both the groups having a scientifically approved composition of Wheat Floor, Poultry Layer Feed and Dried Milk. This food material was purchased from authorized sellers. The ingredients were mixed at a ratio of 4:4:2 with a desired quantity of water to get a dough. Mixed properly to get a homogenous mixture up to the possible extent. The dough was baked in an electric oven.

Smokeless tobacco of a local popular brand was obtained from the market and used throughout the experiment. Ten animals were exposed to tobacco mixed with normal feed, and an equal number of controls were provided normal diet. Tobacco (5%) in powdered form was mixed in the diet after grinding...
properly in an electric mixer. Tobacco was fed during pregnancy from gestational day (GD 1) till parturition. During the observation period, all the animals were periodically weighed and observed for any toxicological signs and symptoms. Twenty Pregnant Swiss albino mice were divided in two groups:

- **Group-A (Experimental)** 10 pregnant mice
- **Group-B (Control)** 10 pregnant mice

From GD 1 until parturition the Group A (Experimental) females (two per cage) were exposed to tobacco mixed with normal feed ad libitum along with clean water and Group B (Control) were exposed to normal feed ad libitum along with clean water. One to two days prior to giving birth (GD 18), dams were separated and housed individually. At birth, each mother/offspring set was maintained in clean filtered air.

Twenty offsprings of two weeks age were randomly selected from both groups, that is, experimental (group A) and control (Group B). Offsprings of both A and B groups were divided into sub groups as follows and were used to assess gross and histological features of liver.

- **Group A-1 (Experimental)** 10 male offsprings
- **Group A-2 (Experimental)** 10 female offsprings
- **Group B-1 (Control)** 10 male offsprings
- **Group B-2 (Control)** 10 female offsprings

The offsprings of both groups were killed by cervical dislocation. The livers were removed, weighed by 0.2 digital meter and fixed immediately in 10% formaldehyde. Livers were kept in labeled jars and were further processed for paraffin embedding. Four micron (um) thick sections were cut & stained with haematoxylin & eosin for light microscopy.

Data was analyzed by SPSS (Statistical Packages for Social Sciences) version16.0. Student paired t-test of two groups was used for comparison. Statistical significance was taken at p< 0.05.

**RESULTS**

**WEIGHT OF THE LIVER OF OFFSPRINGS**

The mean weight of liver in experimental male offsprings was found to be 0.58±0.03 grams and in control group it was 0.78±0.02 grams. Thus the difference in the mean weight of the liver between the experimental and control group was found to be highly significant (p value < 0.001) in both sexes. (Figure # 1)

**HISTOLOGICAL FINDINGS**

The H & E stained slides of livers from the offsprings of both groups were observed and following changes were seen.

The livers from the control group showed normal histological structure of the mice liver (Figure # 2 and 4). In the livers of offsprings from the experimental group, following changes in cell morphology and histo-architecture were observed:

- In 14 out of 20 cases from experimental group, the liver parenchyma showed fatty infiltration (Figure # 3)
- In five of the cases, besides fatty infiltration, there was observed a widespread swelling and ballooning of hepatocytes indicating hydropic degeneration (Figure # 5).

In few cases, the cell swellings were particularly observed around the vessels may be causing vasoconstriction, Kupffer cells hyperplasia and dilated sinusoids.

**Figure-1**: Bar chart showing comparison between the weight (mean value) of the liver of the offsprings of both male and female in the two groups.
DISCUSSION

The use of chewing tobacco in different forms has become quite common in our community especially by females. Smokeless tobacco poses a major risk to women and to the children born to them. This study was designed to observe the use of local tobacco in pregnancy and its effects on the developing liver of the offsprings in Swiss mice.

One of the important findings in our study was a significant decrease in the relative weight of the liver of the offsprings of the experimental group. We are unable to find a comparable study probably due to the fact that the studies we have referred either do not mention the weight of the liver and/or have seen the effects of tobacco consumption by adult animals and have rather reported an increase in weight of the liver mainly accompanied with the fatty degeneration.

Valenca et al have reported no significant change in liver volume with the use of oral nicotine in adult rats. One of the significant findings of our studies was the altered morphology and the histopathological changes in the liver of the offsprings of the tobacco treated mothers. The main findings were the fatty infiltration of liver parenchyma. Besides, we also observed the cell swellings indicating hydropic degeneration. These findings are in agreement with the comparable findings of Avti et al. Our findings are in agreement with some other studies who have observed similar changes in the livers of adult experimental animals treated with either nicotine or nitrosamines.

The effect of nicotine on the liver is the focus of many studies currently, as the nicotine is the principal alkaloid in the tobacco and mainly metabolized in the liver. Further, it is considered that the toxic effects of
cigarette smoking and tobacco use are associated with their nicotine content\textsuperscript{16}. Fatty liver or steatosis refers to a histopathological condition characterized by an excessive accumulation of lipids, primarily triglycerides within hepatocytes\textsuperscript{19}. The results of our study are substantiated by the findings of Nakamoto et al\textsuperscript{11} and Grattagliano et al\textsuperscript{10} who report that both animal data and human studies have shown a link between fatty liver and oxidative stress caused by cigarette smoking and lipid peroxidation.

Through this study we come to the conclusion that the use of smokeless tobacco of local brand during pregnancy has significant effects on the liver of the offsprings of mice, especially fatty changes and hydropic degeneration as well as the reduction in the weight of the liver. However further studies are required to establish these facts with other brands and mixtures of tobacco and by observing dose related changes.

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