Original Article

Systemic effect of arecoline on the gastrointestinal system in oral submucous fibrosis affected wistar rats

Basetty N Rajarathnam[1], Rakesh N[2], Anita Murali[3], Asha ML[1]

[1]Department of Oral Medicine and Radiology, Dr. Syamala Reddy Dental College, Bengaluru, Karnataka, [2]Department of Oral Medicine and Radiology, Faculty of Dental Sciences, [3]Department of Pharmacology, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India

Work attributed to: Department of Oral Medicine and Radiology, M S Ramaiah University of Applied Sciences

Abstract

Background: The intestine plays an important role in the digestion and absorption of ingested food and the elimination of undigested food, microbes, and microbial products. The functional reliability of the intestinal mucosal epithelial cells depends on the organised regulation of the epithelial cells, mucus layer, the intercellular tight junction, host innate and acquired immune response. The mucus layer of the gastrointestinal tract is the first line of innate host defense, essentially because of the secretory products of intestinal cells.

Aim: Present study was conducted to evaluate the effect of arecoline on the gastrointestinal system due to systemic absorption of the drug during the induction period of oral submucous fibrosis (OSMF) in Wistar rats.

Methods: Oral submucous fibrosis was induced by submucosal injection of arecoline in the buccal mucosa. Arecoline hydrochloride at a dosage of 10 mg/kg was injected into the submucosa of right buccal mucosa in experimental animals over a period of 3 months on every alternate day. After which, right buccal mucosa, gastrointestinal tract organs like stomach, large intestine, small intestine and liver were dissected, subjected to histopathological evaluation of the healthy and experimental Wistar rats were subjected to histopathological evaluation.

Results: On histological evaluation, OSMF was seen to affect Wistar rats showed significant changes in oral mucosa, decrease number of goblet cells in the small intestine as well in the large intestine and deranged hepatocytes. These marked changes indicated a definite effect on the gastro intestinal system by arecoline.

Conclusion: The study has highlighted the effect of arecoline due to systemic absorption during the induction process of OSMF in Wistar rats.

Keywords: Arecoline, gastrointestinal system, oralsubmucuous fibrosis, Wistar rats

Address for correspondence: Dr. Basetty N Rajarathnam, Department of Oral Medicine and Radiology, Dr. Syamala Reddy Dental College, Bangalore, Karnataka, India.
E-mail: rajmds06@gmail.com

Submitted: 26-Dec-2021, **Revised:** 19-Feb-2022, **Accepted:** 26-Feb-2022, **Published:** 22-Dec-2022

INTRODUCTION

Areca nut is derived from the seed of Areca catechu. It is grown widely in Asia, Tropical Pacific and East Africa. It is consumed with tobacco, called betel quid or along with betel leaves known as paan.[1,2] Arecoline, arecaidine, guvacoline and guvacine are the four main alkaloids

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_451_21

How to cite this article: Rajarathnam BN, Rakesh N, Murali A, Asha ML. Systemic effect of arecoline on gastrointestinal system in oral submucous fobrosis affected wistar rats. J Oral Maxillofac Pathol 2022;26:598.

of arecanut. These alkaloids undergo glutathione conjugation and form mercapturic acid in rats. Arecoline is metabolised to arecaidine by hydrolysis and N-oxidation in the presence of the enzyme Flavin containing monoxygenases (flavin-containing monooxygenases 1, flavin containing monooxygenases. The major parasympathetic and muscarinic effects of the areca nut are due to arecoline.[3,4] Arecoline (methyl-1, 2, 5, 6-tetrahydro-1-methyl-nicotinate), an alkaloid isolated from A. Catechu was reported to have wide pharmacological activities [Table 1].

These include effects on nervous, cardiovascular, endocrine and digestive systems, and anti-parasitic effects, etc. Apart from the side effects reported, oral submucous fibrosis (OSMF) Oral squamous cell carcinoma are the main oral health considerations.[13]

METHODOLOGY

The present study was carried out in the animal house of the Faculty of Pharmacology, M S Ramaiah University of Applied Sciences, Bangalore, for induction of OSMF in male Wistar rats. Ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC). Registration number 220/PO/abc/2000/CPCSEA and reference number MSRFPH/P/62/2015. Ten healthy Wistar rats weighing approximately about 200 mg were obtained from private animal breeders. Among which, five Wistar rats were grouped as control and five Wistar rats were grouped as experimental animals. All the Wistar rats were kept in clean, hygienic cages and maintained under standard laboratory conditions. The Wistar rats were maintained at a controlled temperature (25°C ± 2°C) with a 12 h light/dark cycle and humidity. They received a standard diet and water ad libitum. Arecoline hydrochloride was procured from Sigma Aldrich Pharmaceuticals. Dosage of 10 mg/kg of freshly prepared arecoline was injected into the submucosa of right buccal mucosa in experimental group Wistar rats over a period of 3 months on every alternate day. At the end of the experiment, the experimental group of Wistar rats was sacrificed, the right buccal mucosa, stomach, large intestine, small intestine and liver were dissected, subjected to histopathological evaluation.

RESULTS

Epithelium shows ortho-keratinizsd stratified squamous epithelium with blunt rete ridges. In connective tissue, normal component of collagen fibres, few inflammatory cells and normal vascularity [Figure 1].

Epithelium shows areas of atrophy and hyperplasia are seen with sub epithelial hyalinisation. In connective tissue, there is an increased density of collagen fibers and inflammatory cells [Figure 2].

There is intact gastric mucosa lined by columnar epithelium. The submucosa shows intact blood vessels. The muscular and serosal layers appear within normal limits [Figure 3].

There is intact gastric mucosa lined by columnar epithelium but, the submucosa shows congested blood vessels [Figure 4].

Histopathology of the large intestine shows intact mucosa lined by columnar epithelium [Figure 5]. The goblet cells appear intact.

Histopathology of the lamina propria shows dense mononuclear inflammatory cells [Figure 6]. The submucosa shows intact blood vessels.

Histopathology of the large intestine shows partially distorted mucosa lined by columnar epithelium [Figure 7]. The goblet cells appear decreased.

Histopathology of the lamina propria shows dense mixed inflammatory cells consisting of lymphocytes and neutrophils [Figure 8].

Section studied from the small intestine shows intact mucosa lined by columnar epithelium [Figure 9]. The villi appear intact.

The lamina propria shows focal mononuclear inflammatory cells [Figure 10]. The submucosa shows intact blood vessels.

Table 1: Studies conducted earlier for evaluation of toxic effect of arecoline on various systems with various doses

Pharmacological effects	Activity	Dose	Study
Antiparasitic Effects	Synergetic effect on killing oncomelania	6.25 μg/mL	Feng *et al*.[5] (1999), Li *et al*.[6] (2000), Yao *et al*.[7] (2001)
Effects on digestive System	Increasing gastrointestinal motility	0.06%	Ni *et al*.[8] (2004)
Effects on nervous System	Inhibitory effective on GABA	8 μM, Guvacine	Lodge *et al*.[9] (1977)
	Inhibiting release of Peripheral catecholamine	0.1 mM	Lim and Kim[10] (2006)
	Sobering up effect	0.25 mg/kg (i.p.)	Sun *et al*.[11] (2005)
Antibacterial and antifungal effects	Anti-Bacillus Proteus	MIC ¼ 0.8 μg/mL	Luo *et al*.[12] (2010)
	Anti-Candida albicans	MIC ¼ 0.8 μg/mL	Luo *et al*.[12] (2010)
	Anti-Bacillus anthracis	MIC ¼ 0.8 μg/mL	Luo *et al*.[12] (2010)

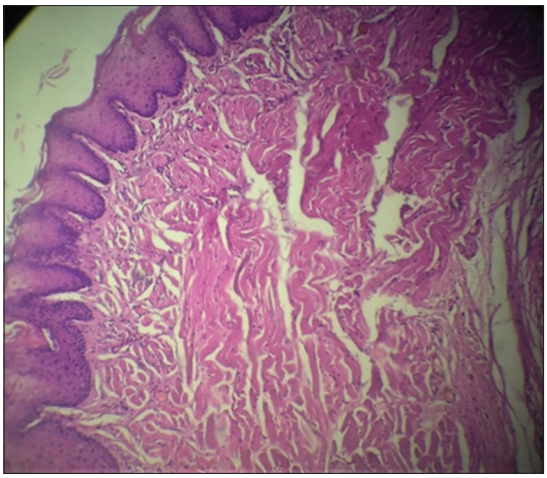

Figure 1: Histopathology of buccal mucosa of healthy wistar rat (H&E, 200X) Epithelium shows ortho-keratinized stratified squamous epithelium with blunt rete ridges. In connective tissue, normal component of collagen fibres, few inflammatory cells and normal vascularity

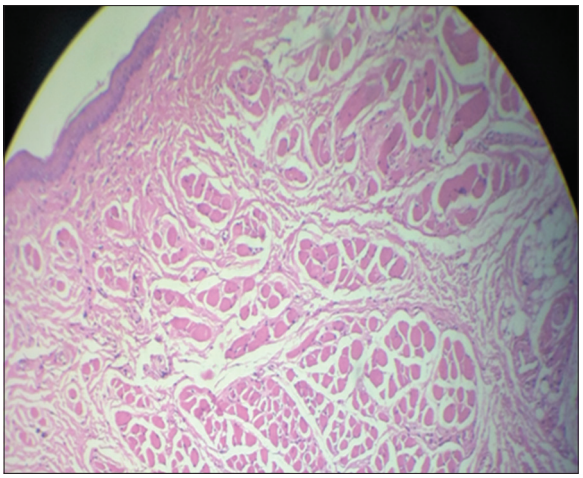

Figure 2: Histopathology of buccal mucosa of oral sub mucous fibrosis induced wistar rat (H&E, 200X). Epithelium shows areas of atrophy and hyperplasia are seen with sub epithelial hyalinisation. In connective tissue, there is an increased density of collagen fibers and inflammatory cells

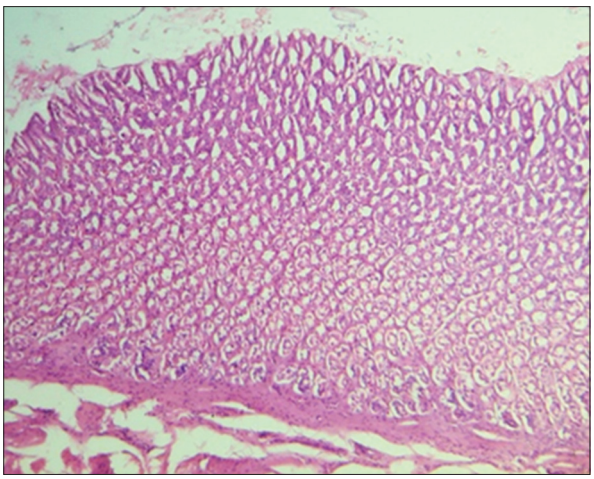

Figure 3: Histopathology of stomach of healthy wistar rat. H&E, 50×. There is intact gastric mucosa lined by columnar epithelium. The submucosa shows intact blood vessels. The muscular and serosal layers appear within normal limits

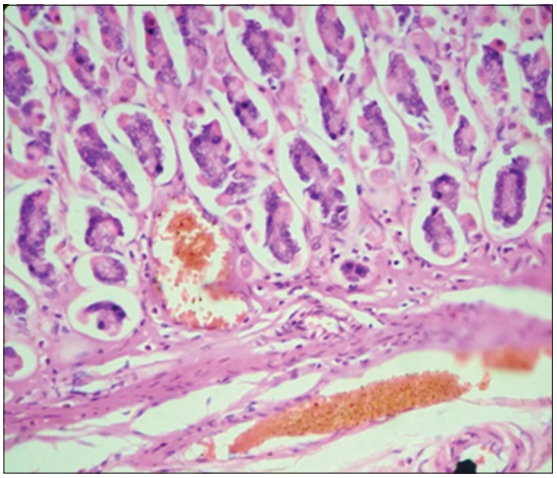

Figure 4: Histopathology of stomach of oral sub mucous fibrosis induced wistar rat (H & E, 200X). There is intact gastric mucosa lined by columnar epithelium but, the submucosa shows congested blood vessels

Section studied from the small intestine shows distorted mucosa with partial loss of villi and lined by columnar epithelium [Figure 11].

The lamina propria shows dense mixed inflammatory cells consisting of lymphocytes and neutrophils [Figure 12].

Section studied shows liver parenchyma with intact architecture [Figure 13].

The perivenular, periportal [Figure 14] and midzonal hepatocytes appear within normal limits. The central veins and sinusoids appear within normal limits.

Section studied shows liver parenchyma with intact architecture [Figure 15]. The perivenular, periportal and midzonal hepatocytes appear within normal limits.

The central veins appear within normal limits and sinusoids appear dilated and congested [Figure 16].

DISCUSSION

Advances in molecular biology have significantly increased the understanding of the biology of different diseases. However, these discoveries have not yet been fully translated into improved treatments for patients with diseases such as cancers. One of the factors limiting the translation of knowledge from preclinical studies to the clinic has been the limitations of *in vivo* disease models

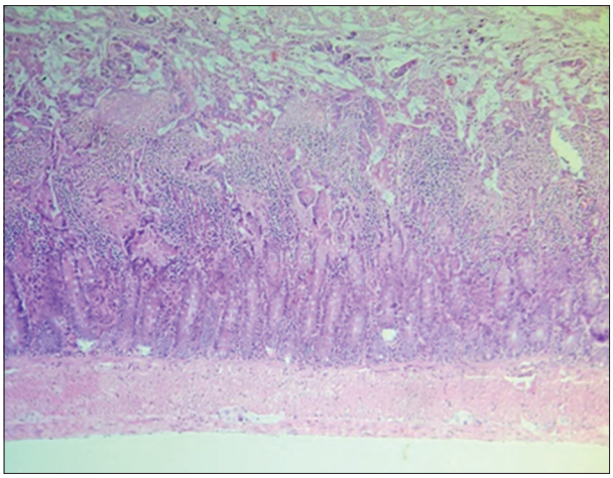

Figure 5: Histopathology of large intestine of healthy wistar rat (H&E, 50X). There is intact gastric mucosa lined by columnar epithelium but, the submucosa shows congested blood vessels

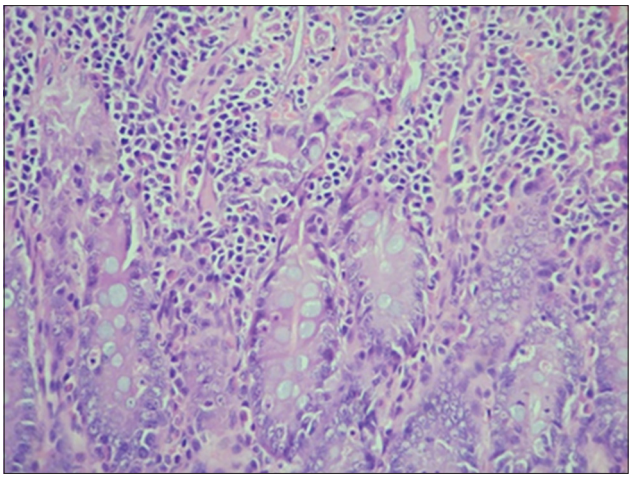

Figure 6: Histopathology of large intestine of healthy wistar rat (H&E, 200X). Histopathology of the lamina propria shows dense mononuclear inflammatory cells. The submucosa shows intact blood vessels

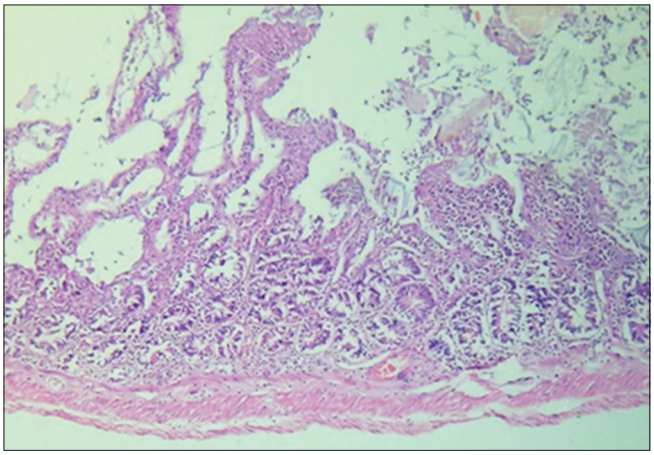

Figure 7: Histopathology of large intestine of oral sub mucous fibrosis induced wistar rat (H&E, 50X). Histopathology of the large intestine shows partially distorted mucosa lined by columnar epithelium. The goblet cells appear decreased

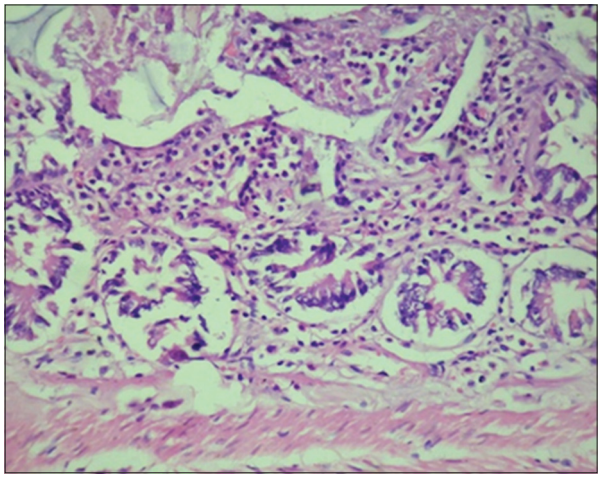

Figure 8: Histopathology of large intestine of oral sub mucous fibrosis induced wistar rat (H&E, 200X). Histopathology of the lamina propria shows dense mixed inflammatory cells consisting of lymphocytes and neutrophils

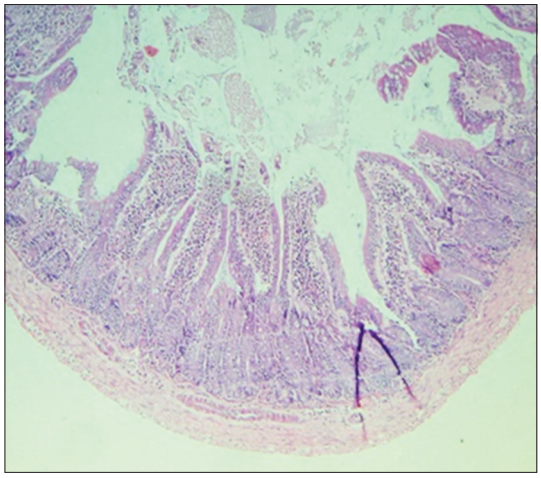

Figure 9: Histopathology of small intestine of healthy wistar rat (H&E, 50X). Section studied from the small intestine shows intact mucosa lined by columnar epithelium. The villi appear intact

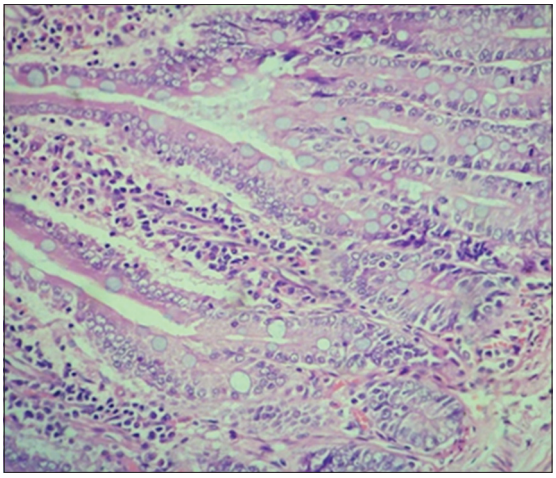

Figure 10: Histopathology of small intestine of healthy wistar rat (H&E, 200X). The lamina propria shows focal mononuclear inflammatory cells. The submucosa shows intact blood vessels

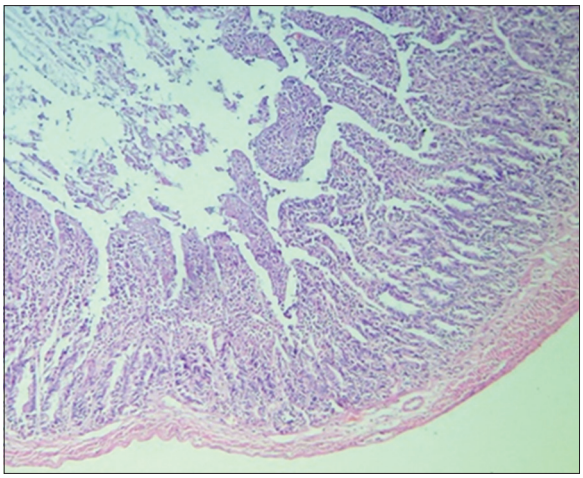

Figure 11: Histopathology of small intestine of oral submucous induced wistar rat (H&E, 50X). Section studied from the small intestine shows distorted mucosa with partial loss of villi and lined by columnar epithelium

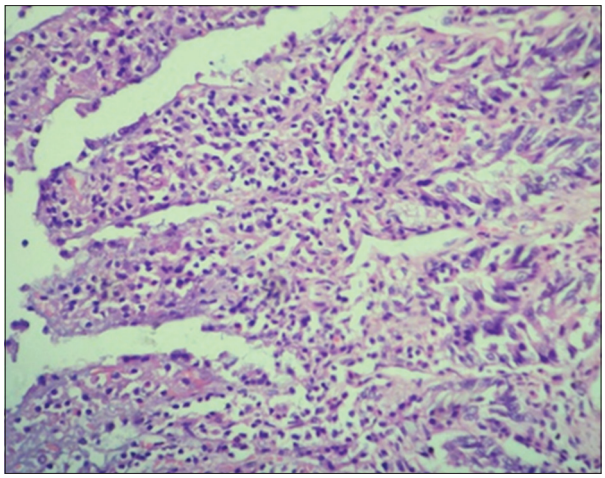

Figure 12: Histopathology of small intestine of oral submucous induced wistar rat (H&E, 200X). The lamina propria shows dense mixed inflammatory cells consisting of lymphocytes and neutrophils

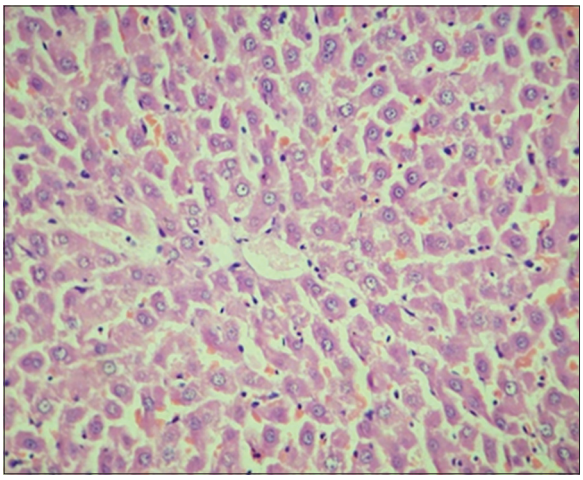

Figure 13: Histopathology of liver of healthy wistar rats (H&E, 200X). Section studied shows liver parenchyma with intact architecture

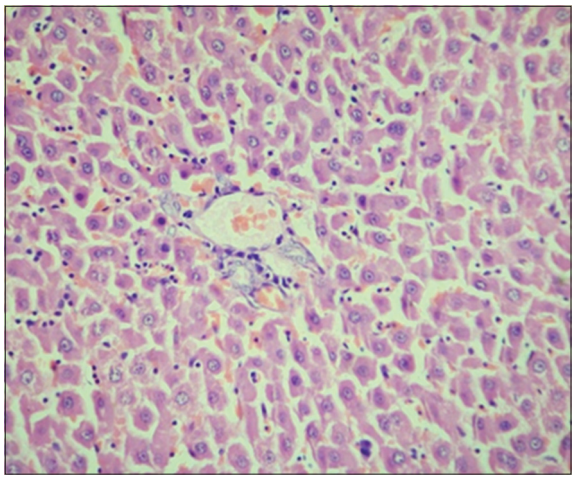

Figure 14: Histopathology of liver of healthy wistar rats (H&E, 200X). The perivenular, periportal and midzonal hepatocytes appear within normal limits. The central veins and sinusoids appear within normal limits

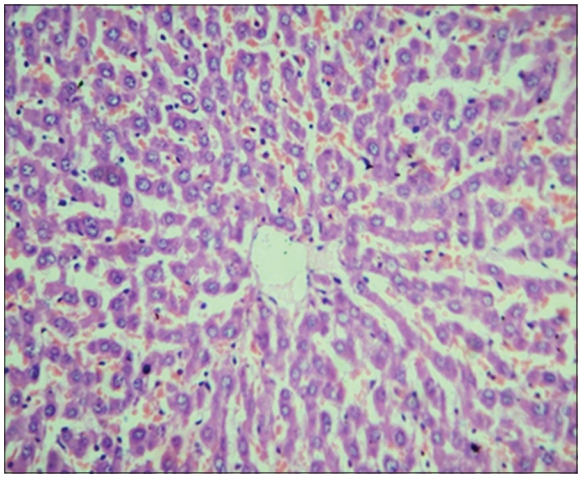

Figure 15: Histopathology of liver of oral submucous induced wistar rats (H&E, 200X). Section studied shows liver parenchyma with intact architecture. The perivenular, periportal and midzonal hepatocytes appear within normal limits

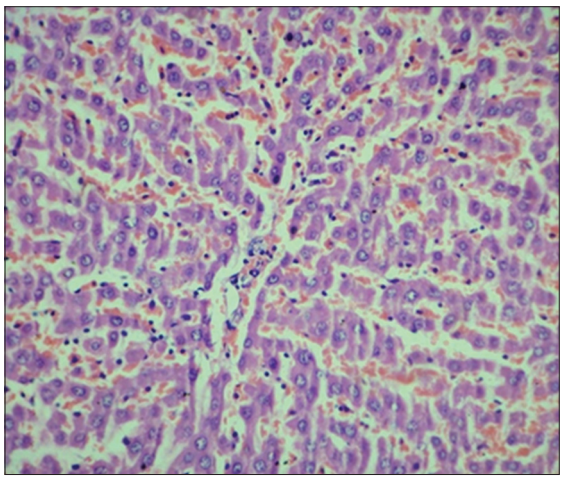

Figure 16: Histopathology of liver of oral submucous induced wistar rats. (H&E, 200X). The central veins appear within normal limits and sinusoids appear dilated and congested

animal experiments remain essential to understand the fundamental mechanisms underlying the onset of malignancies and to discover improved methods to prevent, diagnose and treat diseases. The current excellence of animal care standards is consistent with the experimental conditions needed when conducting cancer research.[14]

The rat (*Rattus norvegicus*) is the most cited animal model used in biomedical research. Compared to mice, rats are bigger, generally more aggressive, and more resistant to various ailments. Sprague-Dawley and Wistar are the two most frequently used rat models.[15]

In our present study, the histological section of buccal mucosa [Figure 2] showed classical features of OSMF which include atrophic epithelium, deposition of collagen in different densities and inflammatory cell infiltration. The findings bear a close resemblance to the characteristic features of OSMF as seen in humans and also as reported in the literature.[16]

Other studies reported in the literature for induction of OSMF in animal models, study on lagomorphs, injection of normal saline, arecanut + tobacco extract O-phenyl phenol over a period of 9 months. Findings: Oral epithelium, treated with arecanut + tobacco has shown progressive changes in thickness leading to ulceration, irregular growth and restricted mouth opening.[17] Study on Swiss albino mice, topical application of gutkha paste (mucoadhesive gel) over a period of 6 months. Findings: considerable induction of OSMF and excellent treatment results on curcumin usage.[18] Study on female BALB/c strain mice, topical application of aqueous extracts of arecanut over a period of 300-600 days. Findings: The changes in the thickness of epithelium –lead to atrophy, fibrosis of connective tissue, increased number of fibroblasts and infiltration of inflammatory cells.[19] Study on male Wistar rats, topical painting of betel nut extract, pan masala extract, tobacco extract and combination of above over a period of 36 months. Findings: OSMF was induced in the rats upon administration of test ingredients for 6 months. Even though changes in the epithelium were observed after 3 months of the administration, the typical features of OSMF were seen after 6 months of the administration.[20]

In our present study, the histopathology of large [Figures 7 and 8] and small intestine [Figures 11 and 12] showed a decrease number of goblet cells in arecoline treated Wistar rats. Contrary to a study conducted by We *et al*.[21] to evaluate 14 days toxicity of arecoline hydrobromide in Wistar rats, found an increase in number of goblet cells. GC (Goblet Cell) dysfunction has been associated with and contributory to multiple diseases including inflammatory bowel disease, cystic fibrosis, asthma, metabolic disorders, Sjögren syndrome, and chronic obstructive pulmonary disease, indicating that GCs are not always innocent bystanders and can be active participants in disease pathogenesis.[22]

Also, arecoline leads to lowering of plasma cholesterol by up to 25% due to inhibition of intestinal acetyl co-enzyme acyltransferase (ACAT) and also pancreatic cholesterol esterase (pACE) enzyme which resulted in reduced cholesterol metabolism.[23] The dose dependent, arecanut chewing habit leads to stimulation of colonic M3 receptors which results in increased gastrointestinal mobility causing a laxative effect. Increased salivation or the sialogogue effect is exhibited due to the presence of AChE inhibitors. These collective effects explain the increased consumption among the rural population in India.[24,25]

In the Present study the histopathology section of the liver [Figures 15 and 16] showed congestion of sinusoids and dilated central veins. It was in agreement with a study conducted by XiaiojuanWe *et al*.[21] in which the liver, hepatocellular damage was evident, including minor granule and vacuolar denaturation, necrosis and interstitial connective tissue proliferation.

Various animal studies have reported that in low doses of arecoline (up to 0.5 mM), it causes G0–G1 cell cycle arrest and DNA damage. In higher doses of arecoline (up to 1 mM), it causes cellular changes like apoptosis and necrosis, eventually leading to deranged hepatocyte cell growth, hepatocellular carcinoma. Arecanut is hepatotoxic causing mixed type of hepatic injury, which is both cholestatic and hepatocellular, and it increases hepatic enzymes namely, serum transaminases and alkaline phosphatase.[26,27]

Jianhong Zhou *et al.,*[28] conducted a study on mice to evaluate the effect of arecoline on hepatic tissues. The author treated mice with arecoline, vitamin C along with vitamin E and a combination of both arecoline and vitamins. He observed there was significant changes in liver enzymes that is, elevated levels of glutamate oxalo-acetate transaminase, serum alkaline phosphatase, glutamate pyruvate transaminase, significantly decreased levels of superoxide dismutase, glutathione-S-transferase, glutathione, catalase.

It is documented by Kaushal *et al.,*[29] that arecoline stimulates choline muscuranic receptor causing increased gastrointestinal peristalsis. Wu *et al.,*[30] evaluated the association between arecanut chewing and the development of liver cirrhosis, hepatocellular cancer. The author concluded that there is 4.25 times increased risk of causing

liver cirrhosis and hepatocellular cancer, the synergistic effect associated with hepatitis B and hepatitis C. Also there was a significant increase in causing esophageal cancers.

A systematic review by Garg *et al*.[31] reported the effect of arecoline by AhR- mediated metabolism in the liver. Arecoline causes downregulation of AhR by neutralizing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. This leads to activation of CYP1A1 and causes liver disorders. The IARC monograph in 2020 reported that arecoline causes chromosomal aberrations in both in-vitro and in-vivo experimental systems. In co-carcinogeneticity studies, there were increased benign tumours of the oesophagus and tongue in rats also, malignant tumours of oesophagus in mice. Arecoline leads to the formation of DNA adducts, more commonly cyclic adducts α, γ hydroxyl-1, N^2 – propanodeoxyguanosine. These adducts were detected in different human biospecimens of hepatic disorders.[32]

CONCLUSION

Recent investigations show that OSMF and oral cancers are the most serious harmful effects of long term use of arecoline and its related agents. The present study highlights the adverse effects of arecoline on GIT. The preclinical, in-vivo study conducted on the animal model showed significant histopathological changes, further which I would recommend clinical and biochemical studies to study the effect of arecoline on GIT. Published literature of in- vivo and in-vitro studies have concerned the linkage between the dosage and the toxicities of arecoline, however, more systematic studies on the pharmacology and toxicology of arecoline should be constructed to obtain the appropriate doses. In addition, more research are needed to be conducted for the evaluation of toxicities of arecoline regarding other aspects and body systems. Collectively, the arecoline possesses a lot of pharmacological effects and also shows some potential side effects. In the future, more investigations are needed to reduce or eliminate the toxicities of arecoline.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Bhisey RA, Boucher BJ, Chen TH, Gajalakshmi V, Gupta PC, Hecht SS. IARC Working Group on the Evaluation of Carcinogenic Risk to Humans: Betel-quid and Areca-nut Chewing and Some Areca-nut-Derived Nitrosamines. Lyon: IARC Pres; 2004.
2. Gupta PC, Warnakulasuriya S. Global epidemiology of areca nut usage. Addict Biol 2002;7:77-83.
3. Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, Gonzalez FJ. A metabolonomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. Chem Res Toxicol 2006;19:818-27.
4. Giri S, Krausz KW, Idle JR, Gonzalez FJ. The metabolomics of (±)-arecoline 1-oxide in the mouse and its formation by human flavin-containing monooxygenases. Biochem Pharmacol 2007;73:561-73.
5. Feng Q, Li GL, Yang Y, Gao J. Studies on the increasing effect componets of mollusicicides in nut of Areca catechu. J Chinese Med Mater 1999;22:572-4.
6. Li GL, Feng Q, Yang Y, Gao J. Studies on the effect increasing components for molluscicides in nut of Areca catechu L. China Journal of Chinese Materia Medica 2000;25:160-2.
7. Yao WX, Xia GJ, Li Y, Fu LY, Deng JP, Wu SY, *et al*. Concentration effect relationship of arecoline on rat portal vein and calcium channel current in guinea pig ventricular myocytes. Chin J Parasitic Dis Control 2001;14:139-41.
8. Ni YD, Wang JH, Wang RJ. Comparative study of the effect of Areca nut and arecoline on gastrointestinal motility. Pharmacol Clin Chin Materia Medica 2004;20:11-2
9. Lodge D, Johnston GAR, Curtis DR, Brand SJ. Effects of the areca nut constituents arecaidine and guvacine on the action of GABA in the cat central nervous system. Brain Res 1977;136:513-22.
10. Lim DY, Kim IS. Arecoline inhibits catechuolamine release from perfused rat adrenal gland. Acta Pharmacol Sin 2006;27:71-9.
11. Sun YP, Han R, Luo J, Chen F, Liang JH. Effects of arecoline on central suppression in mice treated acutely with ethanol. Chinese Journal Of Drug Dependency 2005;14:333-7.
12. Luo SS, Zhang HD, Liu XL, Zhu L. Study on antimicrobial activity of arecoline from betel nut in vitro. Innovational Edition Farm Prod Process 2010;10:47-50.
13. Liu YJ, Peng W, Hu MB, Xu M, Wu CJ. The pharmacology, toxicology and potential applications of arecoline: A review. Pharm Biol 2016;54:2753-60.
14. Vandamme TF. Use of rodents as models of human diseases. J Pharm Bioallied Sci 2014;6:2-9.
15. Jonson M. Laboratory mice and rats. Mater Methods 2012;2:113. doi: 10.13070/mm.en.2.113.
16. Pindborg J, Sirsat S. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1966;22:764-79.
17. Majumdar PK, Pritam Sukul P, Sandhini Sahac S, Verma R, Gupta SS, Nath S, *et al*. Preclinical investigation of pre-malignant oral fibrosis and carcinoma in lagomorphs. Int J Sci Res 2013;2:6.
18. Kumar NS, Bhaskara DV, Rao KP, Pratima S. Pathological observations on the treatment of oral sub mucous fibrosis of curcumin gels in animal models. Pharm Lett 2012;4:919-26.
19. Perera MWS, Gunasinghe D, Perera PAJ, Ranasinghe A, Amaratunga P, Warnakulasuriya S, *et al*. Development of an *in vivo* mouse model to study oral submucous fibrosis. J Oral Pathol Med 2007;36:273-80.
20. Anuradha DC, Hirano S, Devi SC. Studies on the nature and significance of collagen in experimentally induced oral submucous fibrosis in rats. J Clin Biochem Nutr 1999;27:123-30.
21. Wei X, Zhang J, Niu J, Zhou X, Li J, Li B. Evaluation of arecoline hydrobromide toxicity aftera14-day repeated oral administrationin wistar rats. PLoS One 2015;10:e0120165. doi: 10.1371/journal.pone. 0120165.
22. Knoop KA, Newberry RD. Goblet cells: Multifaceted players in immunity at mucosal surfaces. Mucosal Immunol 2018;11:1551-7.
23. Park YB, Jeon SM, Byun SJ, Kim HS, Choi MS. Absorption of intestinal free cholesterol is lowered by supplementation of Areca catechu L. extracts in rats. Life Sci 2002;70:1849-59.
24. Li CB, Yang X, Tang WB, Liu CY, Xie DP. Arecoline excites the contraction of distal colonic smooth muscle strips in rats via the M3 receptor-extracellular Ca2+ influx-Ca2+ store release pathway. Can J Physiol Pharmacol 2010;88:439-47.

25. Gilani AH, Ghayur MN, Saify ZS, Ahmed SP, Choudhary MI, Khalid A. Prescence of cholinomimetic and acetylcholinesterase inhibitory constituents in betel nut. Life Sci 2004;75:2377-89.
26. Lin CF, Shiau TJ, Ko YC, Chen PH, Wang JD. Prevalence and determinants of biochemical dysfunction of the liver in Atayal aboriginal community of Taiwan: Is betel nut chewing a risk factor? BMC Gastroenterol 2008;8:13. doi: 10.1186/1471-230X-8-13.
27. Chang ES, Miao ZF, Lee WJ, Chao HR, Li LA, Wang YF, *et al*. Arecoline inhibits the 2,3,7,8 tetrachlorodibenzo-p-dioxin-induced cytochrome P450 1A1 activation in human hepatoma cells. J Hazard Mater 2007;146:356-61.
28. Jianhong Z, Qi S, Zhirong Y, Jie Z. The hepatotoxicity and testicular toxicity induced by arecoline in mice and protective effects of vitamins C and E. Korean J Physiol Pharmacol 2014;18:143-8.
29. Kaushal M, Mishra AK, Raju BS, Ihsan R, Chakraborty A, Sharma J, *et al*. Betel quid chewing as an environmental risk factor for breast cancer. Mut Res 2010;703:143-8.
30. Wu GHM, Boucher BJ, Chiu YH, Liao CS, Chen THH. Impact of chewing betel-nut (Areca catechu) on liver cirrhosis and hepatocellular carcinoma: A population- based study from an area with a high prevalence of hepatitis B and C infections. Public Health Nutr 2009;12:129-35.
31. Garg A, Chaturvedi P, Gupta PC. A review of the systemic adverse effects of areca nut or betel nut. Indian J Med Paediatr Oncol 2014;35:3-9.
32. Carcinogenicity of acrolein, crotonaldehyde, and arecoline. IARC monographs. November 26, 2020.