Assessment of prophylactic effect of *Senna auriculata* (L.) Roxb. leaves on alcohol-induced pancreatitis in rat model

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Aqueous leaf extract of *Senna auriculata* (L.) Roxb. syn. *Cassia auriculata* (SLEX) is known to possess potential antidiabetic and antioxidant properties. Based on the known correlation between exocrine pancreatic function and endocrine secretory capacity, here, we studied the prophylactic effect of the SLEX on alcohol induced pancreatitis in rats. To induce chronic pancreatitis, the rats were fed with unsaturated fat i.e. corn oil (2.5 mL/kg) along with high dose of ethanol (10.2 g/kg) for 4 wk, and was increased 0.6 g/kg after every 2 days for 1 wk and then 0.6 g/kg after every 4 days for a period of 4 wk. SLEx was orally administered to rats at dose of 400 mg/kg/day for 4 wk. At the end of 4th wk, pancreatic enzymes i.e., α-amyrase, lipase, serum and pancreatic MDA levels were estimated. Pancreatic histopathological studies were also performed. The SLEx significantly reduced the serum levels of α-amyrase and lipase along with significant suppression in serum and pancreatic tissue lipid peroxidation. Histomorphological studies did not show any fatty vacoules in acinar cells of SLEX-treated rats. However, vacoulation was seen in acini of pathogenic control rats. With the results, we conclude that *Senna auriculata* aqueous leaf extract has potential to reduce the ethanol-induced pathogenesis, and it possesses prophylactic effect on alcohol-induced pancreatitis. However, a long term trial is needed to ascertain its therapeutic potential for pancreatitis.

Keywords: Avaram tree, Avari panchaga choornam, *Cassia auriculata*, Matura tea tree, Pancreas, Tanner’s Cassia

Pancreatitis is a painful, inflammatory disorder of the pancreas. Pathophysiology of chronic pancreatitis is heterogeneous and involves a complex cascade of events. Free radical formation plays a major role in pathogenesis of the pancreas after alcohol exposure. The most common cause of chronic pancreatitis is heavy alcohol use.

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The treatment of pancreatitis has remained virtually unchanged for the past five decades and still inadequate. Therefore, effective treatment is an urgent need for pancreatitis. Besides clinical research into gastrointestinal diseases, including the causes and risk factors of pancreatitis, research in the area of drug development is still a priority. In drug development for pancreatitis, plants can play a pivotal role especially, antioxidant and antidiabetic plant extracts having profound effect on normalization of pancreatic damage. In the present study, we explored the well-known medicinal plant *Senna auriculata* (L.) Roxb. syn. *Cassia auriculata*, *Cassia densistipulata* Taub., commonly called Avaram/Matura tea tree or Tanner’s cassia for potential of its leaves in reducing the ethanol-induced pathogenesis as well as the prophylactic effect on alcohol-induced pancreatitis.

Materials and Methods

The leaves of *Senna auriculata* (Fam. Leguminosae) were procured from Nagercoil, Tamil Nadu and a voucher specimen was lodged in herbarium of BGIR, Noida (Voucher specimen no.1605). Crude extract of *S. auriculata* leaves was prepared as per method of Gupta et al.º.

To induce chronic pancreatitis, the rats were fed with unsaturated fat i.e. corn oil (2.5 mL/kg) along with high dose of ethanol for a period of 4 wk daily using the enteral protocol developed by Hiroshi et al.¹º. The ethanol was delivered initially at 10.2 g/kg/day followed by increasing its dose by 0.6 g/kg every 2 days till the end of the 1st wk and then 0.6 g/kg every 4 days till the end of 4th wk. In our earlier dose dependent studies, the effective dose of *S. auriculata* leaf extract was 400 mg/kg body wt. Hence, in the present study too we used the same concentration for prophylaxis of alcohol induced chronic pancreatitis.

The rats were randomized in the following three groups, comprising of 6 rats in each group: Group I, Healthy control; Group II, Rats supplemented with corn oil+ethanol; and Group 3, Rats supplemented with corn oil+ethanol +SLEX. The corn oil, ethanol and extract were administered daily for 30 days using standard orogastric canula. The extract was administered @ 400 mg/kg dissolved in one mL of
distilled water. At the end of the experimental period, blood samples were collected in plain vials from overnight fasted rats by retro-orbital venepuncture technique\textsuperscript{11}. Serum was separated out by centrifugation at 2000 rpm for 10 min and was used for estimation of pancreatic enzymes and serum malondialdehyde (MDA) levels. Thereafter, the animals were euthanized through CO\textsubscript{2} inhalation to excise the pancreas. The pancreatic tissue samples were washed with ice-cold saline and stored at \(-80^\circ\text{C}\) for determination of MDA.

For histomorphological studies, the pancreatic tissue was preserved in 10\% neutral formalin and processed for paraffin embedding and sections were cut at a size of 5 \(\mu\)m, then stained with haematoxylin-eosin for microscopic observation of histomorphological changes. Serum \(\alpha\)-amylase and lipase were measured spectrophotometrically using standard techniques. Lipid peroxides were measured in serum as MDA by the standard method of Satoh\textsuperscript{12}. In pancreatic tissue, MDA was measured by the method of Ohkawa \textit{et al.}\textsuperscript{13}.

**Results and Discussion**

Fig. 1 a and b shows highly significant increase \(P<0.001, 0.01\) in the levels of serum amylase and lipase in the pathogenic control rats, compared to healthy control. Subsequently, serum (Fig. 1 c and d) and tissue MDA levels were significantly increased \(P<0.001\) in pathogenic control rats as compared to healthy control. The microphotograph of pancreatic tissue of healthy rats showed normal architecture of acinar cells and Langerhans islets (Fig. 2a). The sections from pathogenic control rats did not show any major pathological changes. However, vacuolation was seen in some of the acinar cells and cytoplasm was also found frayed (Fig. 2b). The sections from SLEx treated rats showed regular morphology of acini and islets without any signs of vacuolation (Fig. 2c).

Pancreatitis continues to be a therapeutic challenge for physicians and surgeons\textsuperscript{14}. Treatment available is mainly supportive and there are currently no targeted therapies available\textsuperscript{5}. In the present study, the rats developed pancreatitis after administration of high dose of ethanol and corn oil for 4 wk as evident by the increased levels of serum \(\alpha\)-amylase and lipase. Serum \(\alpha\)-amylase and lipase are used as biochemical markers of pancreatitis. The rats those received \textit{Senna auriculata} aqueous leaf extract (SLEx) along with ethanol and corn oil showed significant decrease in
the levels of these pancreatic enzymes in the serum. This shows the protective role of the SLEx against development of alcohol induced pancreatitis.

The pathogenic control rats also showed a significant increase in lipid peroxidation as demonstrated by the MDA levels in serum as well as pancreatic tissue. However, the rats treated with SLEx showed a significant reduction in serum and tissue MDA levels. The tissue MDA levels were found within the normal range in SLEx treated animals. It depicts a strong therapeutic potential of the SLEx as an antioxidant agent in alcohol-induced pancreatitis.

Lipid peroxidation leads to cell membrane damage which in turn responsible for the leakage of pancreatic enzymes into blood stream. Thus, the decreased levels of pancreatic enzymes in the serum of SLEx treated rats can also be justified by reduced tissue MDA levels.

Histomorphological studies of pancreatic sections of rats did not show any major pathological changes, besides some vacoulation and frayed cytoplasm in acinar cells. The pancreatic sections from extract-treated rats showed normal morphology of acini as well as islets. The almost regular morphology of acini without any signs of inflammation and/or necrosis with only a little steatosis suggested that longer treatment with ethanol is required to develop pathological changes in the pancreatic acini. This is further evident as the pancreatic tissue MDA levels in pathogenic group showed only insignificant rise in contrast to a high increase in the respective serum MDA. However, increase in the levels of pancreatic enzymes and serum MDA levels revealed the development of pancreatitis after administration of ethanol for 4 wk.

In conclusion, the results of the study revealed the prophylactic effect of *Senna auriculata* (SLEx) aqueous leaf extract on alcohol-induced pancreatitis. The extract has shown potential in reducing the ethanol-induced pathogenicity which might be due to its antioxidant potential. However, a long term trial is required to elucidate its potential as therapeutic agent for pancreatitis.

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**References**