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Protective effect of *Momordica charantia* in experimentally induced pancreatic atypical acinar cell tumor in male wistar rats through estimation of lipid peroxidation and antioxidant profile

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Abstract
The present study was to estimate the lipid peroxidation ad antioxidant profile in male Wistar rats induced with pancreatic atypical acinar cell tumor and its alleviation by *Momordica charantia* (MC). Ninety six numbers of less than 3 weeks old male albino rats of Wistar strain were randomized into four groups. Rats were administered with a single dose of azaserine at the rate of 30 mg/kg BW intraperitoneally (i.p.) on 21st day of age. Paclitaxel was injected to the azaserine + paclitaxel group (33mg/kg BW) intraperitoneally for 6 weeks after 8 weeks post initiation and aqueous extract of MC (0.34 mL/rat) was administered as oral gavage for 6 weeks after 8 weeks post initiation.
Pancreatic tissue samples were collected in sterile normal saline for lipid peroxidation and antioxidant profile from control and treated rats.
Momordica charantia protected the pancreas from increased lipid peroxidation, increased antioxidant values compared to azaserine + paclitaxel group.

Keywords: *Momordica charantia,* lipid peroxidation, antioxidant profile, pancreatic atypical acinar cell tumor, male wistar rats, paclitaxel

1. Introduction
Pancreatic cancer is an important public health problem. World-wide, carcinoma of the pancreas caused more than a quarter of a million deaths annually, being the 13th most common cancer and the seventh most frequent cause of death from cancer [5, 16].
Given that the majority of cancers occurred in association with smoking, diabetes, pancreatitis, genetic factors, and others and with a growing population worldwide in mind, more cases would be expected in the near future giving further impetus to investigating prevention and treatment strategies to this international issue [9].
Azaserine has been established as a carcinogen for inducing pancreatic tumor in rats. Paclitaxel, which is a commonly used anticancer drug in human malignancies is a complex diterpene having a taxane ring. *Momordica charantia* (MC) (Bitter melon or Bitter gourd) of the family Cucurbitaceae is a common food ingredient in Indian cuisine and is used extensively in alternative medicine. In recent years, rat models were widely used to study the effects of MC juice and its extracts. There were also accumulating reports showing anticancer efficacy and antimutagenic properties of the bitter melon [11, 12].
However, the side effects of paclitaxel therapy has always been a great concern. It includes febrile neutropenia, thrombocytopenia, diarrhoea, sensory neuropathy and fatigue. Owing to these reasons, several other formulations are under trial for providing treatment regimens which enhances the curability and prolongs the survival rate [8].
Hence, the traditional systems of medicine viz. Ayurveda, Siddha and Unani involving the use of plant products in the amelioration of the various diseases were combined in conjunction with conventional medicine to aid in better therapy [9, 4].
Therefore, this study was undertaken to assess the protective effect of aqueous extract of *Momordica charantia* on lipid peroxidation and antioxidant profile in experimentally induced pancreatic atypical acinar cell tumor in rats.

2. Materials and Methods
The study was conducted with the approval of the Institutional Animal Ethical Committee (No. 2345/18/DFBS/IAEC/2016).

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2.1 Experimental animals
Ninety six (96) numbers of less than 3 weeks old male albino rats of Wistar strain were obtained from Laboratory Animal Unit, Madhavaram, Chennai – 51. The standard commercial pellet laboratory animal diet was procured from M/s. Biogen Laboratory Animal Facility, Bengaluru-562 107.

2.2 Chemicals and drugs
Azaserine was obtained from M/s. Sigma Aldrich Inc., St. Louis, USA and stored at -20ºC. The standard drug Paclitaxel was obtained as *gratis* from Cipla Ltd. Mumbai – 83 and stored in dark at room temperature.

2.3 Preparation of aqueous extract of MC
The aqueous extract of MC was prepared from the locally purchased unripened fruits. About 500g of unripened fruits were slit horizontally to remove pulp and seeds and the remaining portions of the fruits were chopped and thoroughly grounded with addition of clean water (approx. 100 mL) using a blender. It was allowed to strain through a muslin cloth into a beaker and the collected extract was filled in centrifuge tubes and centrifuged at 3000g for 30 minutes at 4ºC. The sediment at the bottom was discarded and the clear supernatant was collected in centrifuge tubes and stored at -20ºC until further use.

2.4 Experimental animal management
Three rats each were housed in polycarbonate cages with corn cobb bedding and metal tops. Temperature was maintained between 18ºC and 26ºC and relative humidity ranging between 30 and 70 per cent was maintained. Light and dark cycles were controlled to give approximately a sequence of 12 hours light and 12 hours dark.

Rats were acclimatized for five days prior to the treatment and were observed for clinical signs daily. Clinical examination of the animals were performed on the day of initiation and body weights were recorded for randomization. Rats were randomly distributed to different groups based on their body weight.

2.5 Experimental protocol
Pancreatic atypical acinar cell tumors were experimentally induced with azaserine in 2-3 week old male Wistar rats. Ninety six male Wistar rats were randomized into four groups each of 24 numbers *viz.* control (group I), azaserine (group II), azaserine + paclitaxel (group III) and azaserine + MC (group IV). Azaserine (0.9 per cent sodium chloride as vehicle) was administered as single intraperitoneal (i.p.) dose of 30 mg/kg BW/rat on the 21st day of age to rats of group II, III and IV. Azaserine + paclitaxel group rats was administered 33 mg/kg BW of paclitaxel (in 15 per cent dimethyl sulfoxide) i.p. for 6 weeks after 8 weeks post initiation and azaserine + MC group rats were administered 0.34 mL [8] of aqueous extract of MC as oral gavage for 6 weeks after 8 weeks post initiation. Rats from each group were sacrificed on 24th week after the initiation of the experimental protocol.

2.6 Sample collection
On the 24th week after CO_2 inhalation anaethesia, the rats form all the groups were sacrificed and pancreatic tissue samples were collected in sterile normal saline for lipid peroxidation and antioxidant profile from control and treated rats and stored at -20ºC till the assays were carried out.

2.7 Estimation of lipid peroxidation (LPO)
Lipid peroxidation (LPO) was estimated by the formation of thiobarbituric acid reactive substances (TBARs) by the method of Yagi (1976) [24]. The brilliant pink color formed by reaction of 2-thiobarbituric acid with breakdown product of lipid peroxidation to form malondialdehyde (MDA) was read spectrophotometrically at 532 nm and expressed as μmoles of MDA/g of tissue.

2.8 Estimation of antioxidant profile
Total protein estimation in the pancreas tissue was carried out by the method of Lowry *et al*. (1951). Reduced glutathione (GSH) was estimated by the method of Meron *et al*. (1979) [13], Glutathione peroxidase (GPx) by the method of Rotruck *et al*. (1973) [20], Superoxide dismutase (SOD) by the method of Marklund and Marklund (1974) [11] and Catalase by the method of Caliborne (1985) [1].

2.9 Statistical analysis
The data generated from different parameters of the experimental study were subjected to one- way analysis of variance (ANOVA) test using statistical package for the social sciences (SPSS) software version 20 for Windows.

3. Results and Discussion
Mean (±SE) lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) values in pancreatic tissue of control, azaserine, azaserine + paclitaxel and azaserine + MC treated groups of male Wistar rats are presented in Table 1 and Fig. 1-5.

There was significant (P<0.05) increase in the LPO values in the rats of azaserine, azaserine + paclitaxel and azaserine + MC group when compared to the control group. The LPO values were significantly (P<0.05) higher in the azaserine group when compared to the control group. The LPO values of the azaserine + MC group was significantly (P<0.05) reduced when compared to the azaserine and azaserine + paclitaxel group.

The SOD, CAT, GPx and GST values in the rats of azaserine group was significantly (P<0.05) reduced when compared to the control group. There was significant (P<0.05) increase in the SOD, CAT, GPx and GST values in the rats of azaserine + paclitaxel and azaserine + MC groups when compared to the azaserine group. A significant increase in the SOD, CAT, GPx and GST values in the rats of azaserine + MC group was observed when compared to azaserine + paclitaxel group.

In our study, the lipid peroxidation levels were significantly higher in the azaserine group when compared to the other groups. This was in agreement with the results reported by MacMillan *et al*. (2000) [10] who reported that early development of pancreatic cancer resulted in an overall increase in the production of reactive oxygen species (ROS).

Similarly, the antioxidant status and the SOD levels were significantly altered in pancreatic cancer. This correlated with the reports indicated by Cullen *et al*. (2003) [2], Vaquero *et al*. (2004) [22] that in pancreatic carcinoma specimens, mean values of all antioxidant enzymes were significantly decreased when compared to normal pancreas.

Manganese superoxide dismutase had shown importance in cell cycle regulation and was found to be abnormally low in pancreatic cancer cells. Mohelnikova-Duchonova *et al*. (2011) [14], Durand and Storz (2017) [3] and Wilkes *et al*. (2017) [23] stated that superoxide dismutases (SODs) modulated the

oxidative status of the cell by dismutation of two molecules of O_2 into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). The levels of LPO revealed significant higher levels in the azaserine + paclitaxel group when compared to the azaserine + MC group. This was in accordance with the results of Panis *et al.* (2011) [16] wherein it was reported that paclitaxel treatment in clinical human trials revealed lactate dehydrogenase leakage which enhanced lipid peroxidation and systemic oxidative stress.

The azaserine + MC group showed a significant reduction in the lipid peroxidation values. This was similar to the investigations by Kavitha *et al.* (2011) [6] and reported that it was due to the inherent effects of antioxidant properties in the fruit extracts of *Momordica charantia* which was assessed in oxidative stress induced albino rats and the changes in brain monoamines. MC exhibited a significant quenching effect on *in vitro* lipid peroxidation which indicated its strong antioxidant activity in albino rats. The alteration in the CAT, SOD, GPx and GST levels also agreed with the results of Revathi *et al.* (2012) [19] and Sophia *et al.* (2014) [21].

In our study, lipid peroxidation levels were significantly alleviated in azaserine + MC group when compared to azaserine + paclitaxel group. This was in agreement with the results of Kim *et al.* (2017) [7] who reported that the metabolites present in MC reduced reactive oxygen species in pancreatic cancer cells. Nkondjock *et al.* (2005) [15] reported that the metabolites present in MC were the most efficient singlet oxygen quencher *in vitro* and its dietary intake could be associated with reduced pancreatic cancer risk.

4. Conclusions
This showed that MC treatment significantly reduced the lipid peroxidation status when compared to paclitaxel therapy which might symbolize cancer chemoprevention using antioxidant approaches.

5. Acknowledgments

Table 1: Mean (±SE) values of pancreatic lipid peroxidation and antioxidant values of pancreatic tissue in the control, Azaserine, Azaserine + Paclitaxel and Azaserine + MC treated male Wistar rats

GROUP	LPO (MDA/g of tissue)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	GST (U/mg protein)
Control	2.07[a] ± 0.16	5.78[d] ± 0.19	18.09[d] ± 0.24	8.08[d] ± 0.14	7.33[d] ± 0.12
Azaserine	7.69[d] ± 0.15	1.82[a] ± 0.11	3.89[a] ± 0.28	2.95[a] ± 0.14	2.43[a] ± 0.07
Azaserine + Paclitaxel	6.57[c] ± 0.12	3.29[b] ± 0.08	13.30[b] ± 0.12	4.54[b] ± 0.14	3.34[b] ± 0.11
Azaserine + MC	5.58[b] ± 0.09	4.28[c] ± 0.07	14.76[c] ± 0.13	5.75[c] ± 0.09	4.35[c] ± 0.09

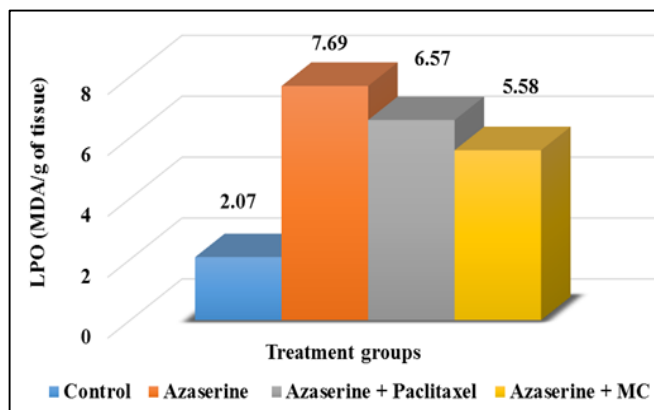Means with same superscript within a column do not differ from each other (P<0.05)

Fig 1: Comparative mean lipid peroxidation (MDA/g of tissue) values of control and treated groups of male Wistar rats
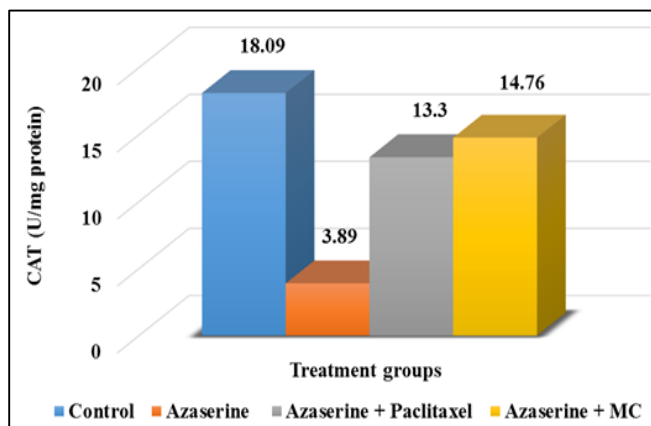

Fig 3: Comparative mean catalase (U/mg protein) values of control and treated groups of male Wistar rats
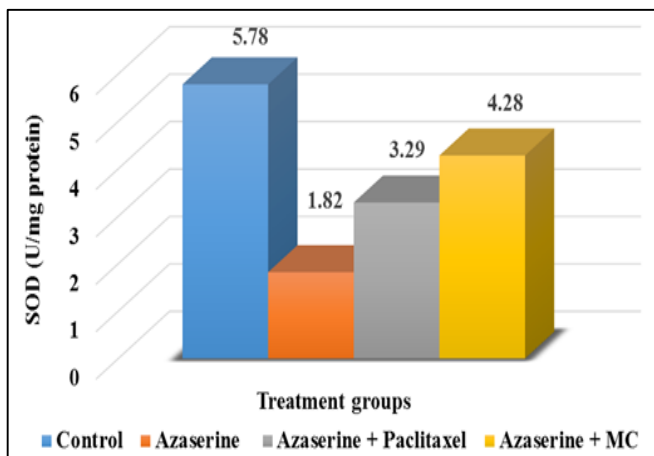

Fig 2: Comparative mean superoxide dismutase (U/mg protein) values of control and treated groups of male Wistar rats
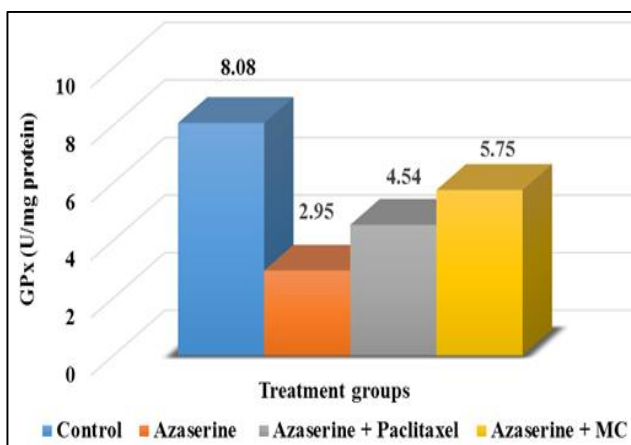

Fig 4: Comparative mean glutathione peroxidase (U/mg protein) values of control and treated groups of male Wistar rats
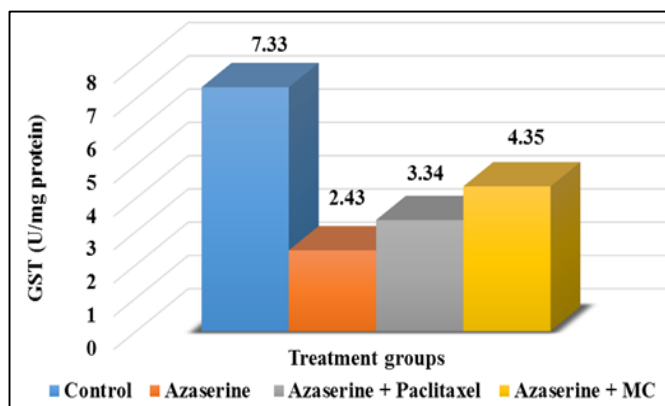
Fig 5: Comparative mean glutathione – S - transferase (U/mg protein) values of control and treated groups of male Wistar rats

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