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Ameliorative effect of phytoconstituents of *Moringa oleifera* L. on body weight in cerulein induced acute pancreatitis in Swiss albino mice

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Abstract

The present study was conducted to evaluate the ameliorative effect of phytoconstituents of *Moringa oleifera* in cerulein induced acute pancreatitis in Swiss albino mice. In this study, the model with high efficiency and huge reproducibility, cerulein induced Acute pancreatitis model was used. Supramaximal doses of cerulein was used for induction of the disease. The average body weights (g) of mice were not significantly altered among the groups. This could be attributed to activation of NRF2 signalling, with its potential power of isothiocyanate moringin and also polyphenols.

Keywords: Acute pancreatitis, cerulein, Swiss albino mice- *Moringa oleifera* L. phytoconstituents

Introduction

Acute pancreatitis (AP), an inflammatory condition of the exocrine pancreas that occurs suddenly and may be mild or even fatal to the organism and is associated frequently with significant morbidity and mortality. Global incidence of the disease has increased in the past several decades and is the leading cause of hospitalisation under non-malignant gastrointestinal disorders (Yadav and Lowenfels, 2013) [1]. AP, a non-infectious endogenous pancreatic injury manifests as local, peripancreatic inflammation and causes distant organ damage like liver injury and acute respiratory syndrome through systemic inflammatory response syndrome (Zyromski and Murr, 2003) [2].

Cerulein, a decapeptide molecule and a naturally occurring pancreatogenic agent is structurally and functionally similar to cholesterol (Angelucci *et al.*, 1974) [3]. Cerulein was discovered in the Australian green tree frog (*Litoria caerulea*) by Italian and Australian researchers in late 90's and is known to be used for inducing both acute and chronic pancreatitis with high success rate.

Supramaximal doses of cerulein in a biological entity responsible for premature activation of trypsinogen with subsequent auto activation of enzymes in the acinar cells that results in over secretion of amylase and lipase enzymes and lead to autodigestion of pancreas. This peripancreatic injury will inturn lead to inflammation of pancreas and release of pro inflammatory cytokines which gain access to systemic circulation and lead to distant organ damage with rise in liver enzymes. (Gallagher *et al.*, 2004) [4].

Moringa oleifera Lam. is a tree that grows widely in many tropical and subtropical countries. *Moringa* leaves are mostly used in culinary purpose because of its high nutritive value with the balance of vitamins, minerals, amino acids and fatty acids. *Moringa* leaves are highly rich in having the antioxidant compounds such as carotenoids, flavonoids, phenolics and ascorbic acid and even act as rich source of β -carotene, calcium, protein vitamin C, and potassium (Dillard and German, 2000) [5], Siddhuraju and Becker, 2003) [6]. The plant phytochemicals present are mainly of major types which includes polyphenols, carotenoids, alkaloids and terpenoids (Ma *et al.*, 2020, and Prabu *et al.*, 2019) [7, 8].

Materials and Methods

The present experimental study was conducted in male Swiss albino mice of uniform age (about 6-8 weeks), which were procured from Jeeva Life Sciences, Hyderabad. Sterile mice feed in the form of pellets was purchased from M/s. Jeeva Life sciences, Hyderabad.

All the drugs used in the study were purchased locally in Hyderabad. *Moringa oleifera* Lam. leaves were thoroughly washed with tap water to remove any waste and dust particles. The excess water was removed by pressing with a paper towel gently and allowed to dry under shade for 3-4 days until constant weight is achieved. Once the samples were dried they were ground to semi powder form using a commercial grinder and were stored in an airtight polythene bags in the refrigerator at 4 °C for further analysis. Later, ethanolic extracts were prepared following the established protocol.

A total of 40 male Swiss albino mice of 6-8 weeks of age, weighing between 22-30 g. were obtained from Jeeva Life

sciences, Hyderabad. The animals were kept in polypropylene cages and maintained with 12 hrs. dark/light cycle under hygienic conditions having ambient temperature (22–24 °C) for acclimatization (1 week) prior to the beginning of the experiment. Animals were placed on commercial standard pellet feed and provided water *ad libitum* throughout the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee, College of Veterinary Science, Rajendranagar, Hyderabad. All the groups were maintained as per the following treatment schedule for 7 days. The mice were randomly divided into 5 groups consisting of 6 mice in each group with the following schedule.

Experimental Design

Group-1	Normal control (NC)/Sham- normal saline for 7 days.
Group-2	<i>Moringa oleifera</i> control/ <i>per se</i> @ 400 mg/kg b.wt. through oral route.
Group-3	Cerulein control (CC)/Positive control/Disease control (DC) received 6 consecutive hourly intraperitoneal injections of cerulein @ 50 µg/kg b.wt. on 7 th day.
Group-4	<i>Moringa oleifera</i> control low dose @ 200 mg/kg b.wt. + cerulein (Pre-treatment with <i>Moringa oleifera</i> control low dose @ 200 mg/kg b.wt. + 6 consecutive hourly intraperitoneal injections of cerulein @ 50 µg/kg b.wt. on 7 th day).
Group-5	<i>Moringa oleifera</i> control high dose @ 400 mg/kg b.wt. + cerulein (Pre-treat (Pre-treatment with <i>Moringa oleifera</i> control high dose @ 400 mg/kg + 6 consecutive b.wt. + 6 hourly intraperitoneal injections of cerulein @ 50 µg/kg b.wt. on 7 th day).

Mice body weights were measured and recorded on the 7th day.

Statistical analysis

The results of the experiment are presented as mean ± SEM (standard error of the mean) where multiple determinants were considered and comparison among groups was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test using GraphPad prism scientific software version 5.0. P- value < 0.05 was considered as significant.

Results and Discussion

Average body weight

The average body weight (g) in group 3 (27.83±1.38) was not significantly different from group 1 (28.00±1.69). Similar results were noticed in treatment groups 4 and 5 (29.25±1.60 & 27.75±0.48 respectively). Further, no significant difference was observed in the average body weights of mice among groups 1 and 2 (Table 1).

Table 1: Average body weight (g) in different groups of mice

Group	Day 7
1. Normal Control (NC)	28.00±1.69
2. <i>Moringa</i> Control	30.75±0.85
3. Cerulein Control (CC)	27.83±1.38
4. Cerulein + <i>Moringa</i> Low Dose	29.20±1.60
5. Cerulein + <i>Moringa</i> High Dose	27.75±0.48

Values are Mean±SE (n=6); One-way ANOVA with Tukey's Multiple Comparison Test (GraphPad Prism version 5.0). Mean±SE without any superscripts were non-significant.

Discussion

The average body weights (g) of mice were not significantly altered among the groups. This could be attributed to its high nutritional and pharmacological properties which aids in activation of NRF2 signalling with its potential power of isothiocyanate moringin and also the miracle power of polyphenols which all help in activation of the NRF2 signalling pathway (Manjunath *et al.*, 2023) [9]. The anti-inflammatory effect exhibited by *Moringa oleifera* is by reducing the activity of proinflammatory enzymes by its

quercetin (Gomez *et al.*, 2023) and kaempferol (Singh *et al.*, 2009) [10, 11].

Conclusion

It is evident from the investigation that there was no significant differences recorded in body weights of the mice across the treatments which might be attributed to presence of terpenoids, flavonoids, phenolic compounds, quercetin, kaempferol and isothiocyanates which are known to have anti-inflammatory potential that might be attributed for maintenance of the body weight in *Moringa oleifera* treated mice. Further, investigations are required to delineate the actual role of these phytoconstituents at molecular level

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