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EFFECT OF HYDROGEN SULFIDE SYNTHESIS ENZYME INHIBITION BY HISTOPATHOLOGY IN PSYCHOLOGICAL STRESS-INDUCED ULCERS IN MICE

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ABSTRACT

Objective: Effect of Hydrogen Sulfide Synthesis Enzyme Inhibition by histopathology in Psychological Stress-Induced Ulcers in Mice. Material and methods: Acute ulcers were induced in Swiss albino mice by using stress. The experimental groups consists of the following 2 groups: sender, responder groups. Sender animals received a foot shock of 10 sec duration at intervals of 50 sec for 3 hour. The electrical current for the shock 1.6 mA to 2.0 mA per hour for 3 day sender animals are changed daily. On day-3, after completing the foot-shock period, the responders were sacrificed, and their stomach were removed. Drug (O-carboxy methyl hydroxylamine hemihydrochloride) were administered intraperitoneally with low (2mg/kg), medium (5 mg/kg), high (10 mg/kg) dose daily for 3 days respectively, 30 min before the shock period. Resulted: The proper development of ulcer with an average ulcer index of 5 mg/kg was found to be 33.42 which is significant with respect to control group. CBS at a dose of 5 mg/kg significantly increased the ulcer formation, which was corroborated by histopathology studies. Conclusion: The present study that observed inhibition of H_2S synthesis blocker exacerbated the psychological stress induced ulcer implicacy an important role of H_2S in regulator of stress.

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INTRODUCTION

Stress is defined as an acute threat to the homeostasis of an organism. Stress evokes adaptive responses that serve to defend the stability of the internal environment [1]. Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue. The peptic ulcers are erosion of lining stomach or the duodenum The peptic ulcers are erosion of lining stomach or the duodenum. The two most common types of peptic ulcer are called "gastric ulcer" and "duodenal ulcer" [2]. Functional gastrointestinal disorders (FGID) are associated with increased anxiety and depression and have a large negative impact on the quality of life. Three of the most common types of FGID are irritable bowel syndrome (IBS), functional abdominal pain, and functional dyspepsia [3].

Hydrogen sulfide along with carbon monoxide and nitric oxide is an important signalling molecule and it is involved in various physiological activities associated with vascular contractility, pro- and anti-inflammatory activities [4]. Endogenous hydrogen sulfide is produced from L-cysteine by cystathionine gamma lyase and cystathionine beta synthase (CSE and CBS), and 3-mercaptopyruvate sulfurtransferase (3- MST) [5].

H_2S is implicated in maintaining GI mucosal defence. Administration of inhibitors of CSE or CBS over the course of a week is reported to result in significant inflammation along the length of the GI tract. The important role play of H_2S in modulated GI inflammation, repair and disuss the potential use of H_2S release drug in treatment of the inflammatory bowel disease and NSAIDs. The H_2S synthesis was found as specify regulated sites of mucosal ulceration and reduce the rates of inactivation of H_2S [6].

O-CHH is used like a nonspecific inhibitor for CBS. This is also effect endogenous H_2S level by inhibiting the activities of CBS and / or CAT [7]. AOAA is soluble in physiological saline solution and the pH was adjusted to 7.4 by using a NaOH. AOAA was tested on membrane potential and spontaneous motility. AOAA did not depolarize on the smooth muscle cells and produced a transient increase in motility [8]. Previous studies done in our laboratory indicate an important role of H_2S in pathophysiology of stress induced ulcers in mice. It was demonstrated that L-Cysteine a H_2S doner produced marked improvement in the prognosis of stress induced ulcers [5]. O-CHH is also called AOAA.

MATERIAL AND METHOD

Material

The drug O- carboxy methyl hydroxylamine hemihydrochloride have been used for study (Sigma Aldrich USA company). A communication box apparatus was used to expose the mice to conditioned emotional stimuli (CES).

Method

All experimental procedures and protocols used in this study were revised and approved by the Institutional animal ethical committee (IAEC) of college of pharmacy IPS Academy, Indore, constituted under committee for the purpose of control and Supervision on Experiment on Animals (CPCSEA).

The experimental groups consists of the following 2 groups: sender group, responder group. Sender animals received a foot shock of 10 sec duration at intervals of 50 sec for 3 hour. The electrical current for the shock is increased step-wise from 1.6 mA to 2.0 mA per hour. Responders were exposed daily to the emotional responses of sender animals, 3h per day for 3 day sender animals are changed daily to naive mice to prevent a reduced emotional response to foot shock based on adaptation or learned helplessness due to repeated exposure. Both sender and responder animals were placed individually in each compartment of the communication box 15 min before beginning the shock period. On day-1, responder animals were returned to their home cages after the 3hr foot shock period.

On day-2, after the completing foot shock period, they were transferred to metal cages and were housed in the cages with 4 animals per cages under food deprivation condition. Food yoked control animals were maintain to the metal cage during foot shock period under the aggregated housing condition (4 animal each) and they were returned to the home cages after the foot-shock period. From beginning of the day-2 experiment, they were maintained in the metal cages under the aggregating housing.

On day-3, just after the completing the foot-shock period, the responders were sacrificed by chloroform, and their stomach were removed. The stomach were visually inspected for lesions. Drug (O- carboxy methyl hydroxylamine hemihydrochloride) were administered intraperitoneally with low (2mg/kg), medium (5 mg/kg), high (10 mg/kg) dose daily for 3 days respectively, 30 min before the shock period.

Method of sacrifice

- Cervical dislocation method.

Method of gastric fluid collection:

- Mice was sacrificed and dissected.
- Pylorus portion was tied through a thread.
- Stomach was isolated and pylorus portion was cut.
- A syringe filled with distilled water was passed through oesophagous and gastric fluid oozed out from pyloric section.

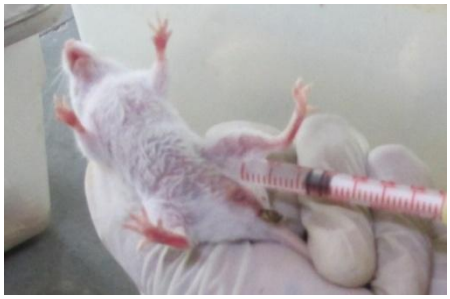


Fig 1 Administration of CHH intra-peritonially.

Experimental Design

Group I : Control
Group II: O- carboxy methyl hydroxylamine hemihydrochloride (2 mg/kg)
Group III: O- carboxy methyl hydroxylamine hemihydrochloride (5 mg/kg)
Group IV: O- carboxy methyl hydroxylamine hemihydrochloride (10 mg/kg)
Group V: Dizepam

Histopathology analysis

Histological studies were performed by taking a small piece of tissue, including ulcers, were embedded in paraffin and sectioned at 5µm in an automated microtome. Haematoxylin and eosin staining was done and tissue was observer under microscope.

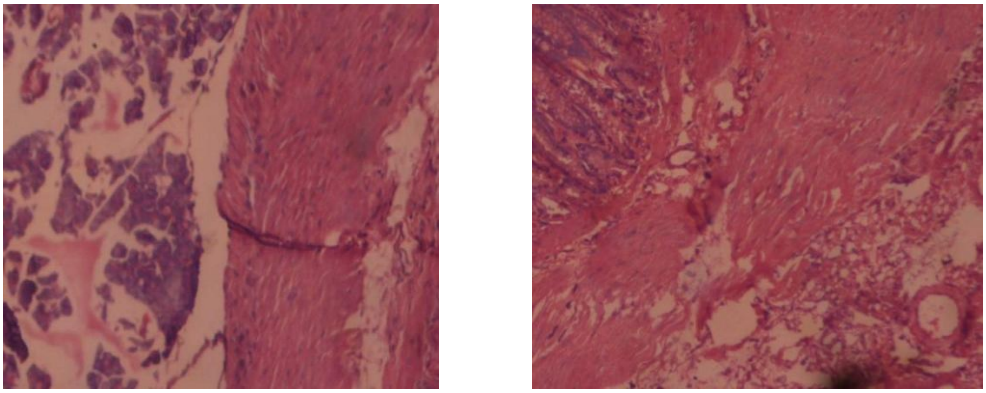
RESULT

Effect of O- carboxy methyl hydroxylamine hemichloride administration on the histopathology

The results of haematoxylin and eosin staining are shown in fig. The control mice exhibited marked mucosal damage. The mucosal lining appears to be completely eroded in the lesion area. O-carboxymethyl hydroxylamine hemichloride administration appears to have preserved the intestinal preserved. Photomicrographs of representative tissue section are shown in

Control group

Figure 2 shows normal mucosal tissue in which the intestine was cut and then placed in formalin solution (10%) which when examined through microscope showed no ulcer. Histopathological analysis showed beginning of necrosis, sloughing manifested as shortning of villi with infiltration of inflammation cell in mucosa at 10x. On 40x showed ulcers having extensive necrosis, sloughing of fibrous tissue proliferation changes and infiltration of mononuclear cells.



A **B**

Fig 2 Photograph (Histopathology) of duodenum of the mice of control group.

A) Histologic section (10x) acute ulcer shows beginning of necrosis, sloughing manifested as shortning of villi with infiltration of inflammation cell in mucosa.
B) Histologic section (40x) acute ulcer shows extensive necrosis, sloughing of fibrous tissue proliferation changes and infiltration of mononuclear cells.

Low dose of CBS antagonist (1 mg/kg)

Fig 3 shows test group section in which effect of CBS antagonist of low dose (1 mg/kg) on psychological stress induced ulcer was seen and that did not show any change in ulcer.

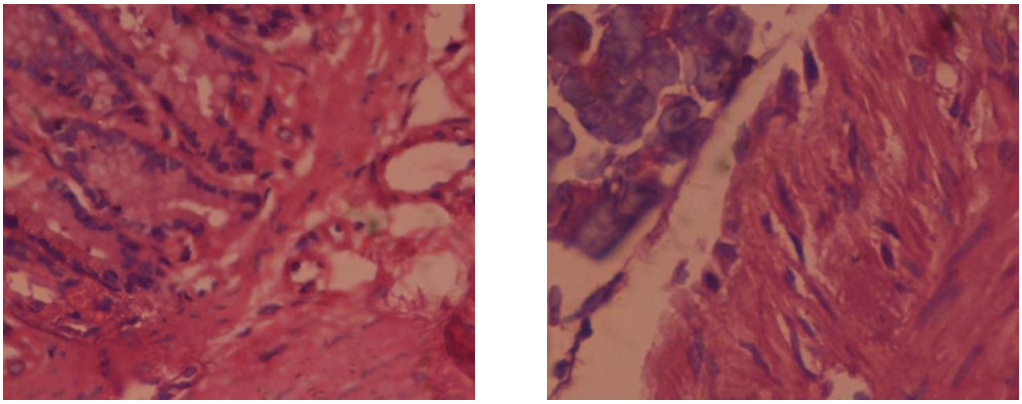


Fig 3 Photograph (Histopathology) of duodenum of the mice of Low dose of CBS antagonist.

A) Histologic section (10x) of acute ulcer shows beginning of necrosis, sloughing manifested as shortning of villi with infiltration of inflammation cell in mucosa.
B) Histologic section (40x) acute ulcer shows extensive necrosis, sloughing of fibrous tissue proliferation changes and infiltration of mononuclear cells.

Medium dose of CBS antagonist (5mg/kg)

Fig 4 shows test group section effect of in which effect of CBS antagonistof medium dose (5mg/kg) on psychological stress induced ulcer was seen and that showed change i.e exacerbation in ulcer.

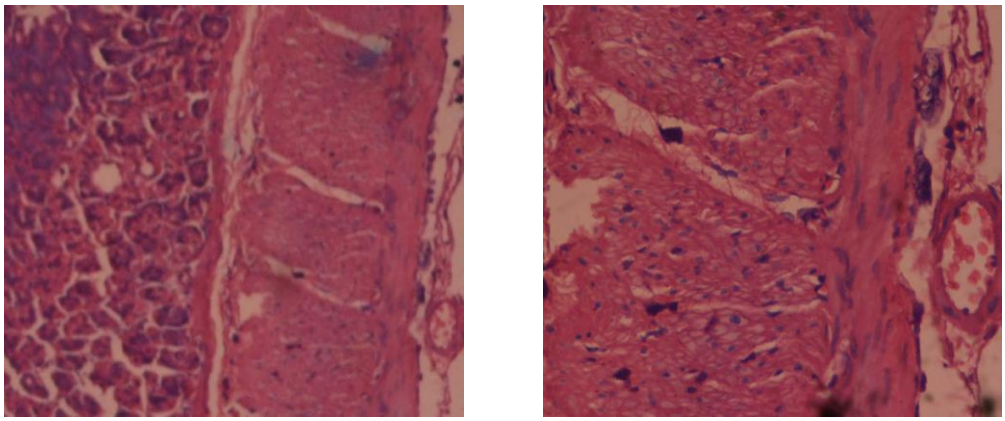


Fig 4 Photograph (Histopathology) of duodenum of the mice of Medium dose of CBS antagonist.

A) Histologic section (10x) of acute ulcer shows beginning of necrosis, sloughing manifested as shortning of villi with infiltration of inflammation cell in mucosa.
B) Histologic section (40x) acute ulcer shows extensive necrosis, sloughing of fibrous tissue proliferation changes and infiltration of mononuclear cells.

CONCLUSION

The present study that observed inhibition of H_2S synthesis blocker exacerbated the psychological stress induced ulcer implicacy an important role of H_2S in regulator of stress induced effects on physiology.

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