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DIPYRIDAMOLE ATTENUATES THE GASTRIC INJURY IN ADULT AND AGED FEMALE RATS

S. Hassan[1]*, A. Rashad[2] and G. H. Heeba[1]

[1]Minia University, Faculty of Pharmacy, Department of Pharmacology and Toxicology, El- Minia, Egypt.
[2]Assiut University, Faculty of Medicine, Department of Pharmacology, Assiut, Egypt.

***Corresponding Author: Dr. S. Hassan**
Minia University, Faculty of Pharmacy, Department of Pharmacology and Toxicology, El- Minia, Egypt.

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ABSTRACT
Age is one of the important factors that affect the drug effects. Gastric ulcer is a common disease especially in elderly. Patients, especially elderly, who are treated with anti-platelet drugs may be exposed to a risk of gastric ulcers. Dipyridamole (DPY) is a platelet inhibitor that is primarily recognized as an antithrombotic agent. The aim of this study is to evaluate the age-dependent effects of DPY against cold restraint stress (CRS)-induced gastric ulcer in adult and aged rats. In addition, the possible mechanisms occupied in the effects were evaluated. DPY was suspended in 2% arabic gum solution and orally administered at a dose of 10 mg/kg once daily for 2 weeks and the last dose was administered 30 min before CRS. DPY exhibited gastroprotective effects in both adult and aged rats as evidenced by significant decreases in ulcer index (U.I). Both adult and aged DPY treated groups showed significant decrease in gastric mucosal malondialdehyde (MDA) concentration and myleoperoxidase (MPO) activity with concomitant increase in gastric mucosal content of reduced glutathione (GSH), nitric oxide (NO), prostaglandin E_2 (PGE_2) levels and gastric juice mucin concentration compared to CRS-induced gastric ulceration in adult and aged rats, respectively. This protective effect of DPY was confirmed by gastric histopathological examination. In conclusion, DPY protected both adult and aged rats' gastric mucosa against CRS-induced gastric ulceration possibly through enhancement of gastric mucosal defense, anti-oxidant and anti-inflammatory activities. Accordingly it is concluded that DPY represents a suitable antiplatelet agent for patients who are at risk of gastric ulcers especially elderly patients.

KEYWORDS: Dipyridamole- Gastric ulcer -Aged rats- Antioxidant - Anti-inflammatory.

INTRODUCTION
The most common stomach diseases in elderly individuals are atrophic gastritis and peptic ulcer disease.[1] Peptic ulcers in elderly are caused by the use or overuse of nonsteroidal anti-inflammatory drugs (NSAIDs).[2]

Peptic ulcer has been associated with multipathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. acid, bicarbonate, pepsin) and maintenance of the mucosal integrity through the endogenous defense mechanism (e.g. defensive mechanisms of mucus, mucosal turnover and blood supply.[3] The enhancement of the mucosal defense mechanisms can be achieved through increasing mucus production, stabilizing the surface epithelial cells or interfering with the PG synthesis.[4]

Reactive oxygen species (ROS) play a major role in oxidative damage of mucosa produced in all types of ulcer including stress-induced gastric ulcer. This is due to enhancing lipid peroxidation and attenuation of mucosal antioxidative mechanism.[5]

Antiplatelet drugs are used for reduction in the risk of thromboembolic events, asymptomatic or symptomatic.[6] Daily administration of aspirin in low-dose is recommended for primary prevention of myocardial infarction and stroke in higher-risk patients.[7] The use of aspirin in patients can lead to serious gastrointestinal mucosal damage.[8]

Dipyridamole has classical anti-platelet and vasodilator actions. In addition, it exhibits anti-inflammatory, anti-oxidant, anti-proliferative and endothelial protective potentials.[9]

The aim of this study is to investigate the age-dependent effects of DPY, on gastric ulcer induced by CRS in adult and aged female rats. In addition, an attempt to evaluate the possible mechanisms involved in this effect was evaluated.

MATERIALS AND METHODS

Drugs and Chemicals

Dipyridamole was obtained from Boehringer Ingelheim pharmaceutical company, Germany. Enzyme-linked immunosorbent assay (ELISA) kit for assessment of gastric mucosal prostaglandin E_2 (PGE_2) was provided by Elabscience Biotechnology Co., Ltd (Hubei, China). All other chemicals were of analytical grade and were obtained from El-Nasr pharmaceutical company, Egypt.

ANIMALS

Female adult (weighing 170-200 gm) and aged (weighing 250-300 gm) Wister albino rats were used in this study. Animals were provided from animal house, Assiut University. Female rats were selected in this study to sure their aging as measured by the stoppage of estrus cycle. The chosen animals were housed in plastic cages with good aerated covers at 25°C ± 0.5°C as well as 12 h light/dark cycles. Rats received a standard diet *ad libitum* with free access to water. Animals were left to acclimatize to the environment for one week prior the experiments. The protocol of this study was approved by the Research Ethics Committee of Faculty of Pharmacy, Minia University, Egypt.

INDUCTION OF GASTRIC ULCERATION

Rats were deprived of food for 24 hours prior to the experiment in mesh-bottomed cages to minimize coprophagia but allowed free access to water except for the last hour before the experiment. Pyloric ligation was carried out according to the method of Morsy and his coworkers[10], directly before CRS, to enable collection of the gastric juice. Peptic ulcer was induced in rats by CRS according to the method of Till and his coworker.[11] All experiments were performed during the same time of the day to avoid diurnal variations of putative regulators of gastric functions.

EXPERIMENTAL DESIGNS

Two main groups of rats were used in this study, adult and aged type. In each group, three subgroups (8 animals each) were employed and included:

1- Normal control "-ve control".

2- CRS- induced gastric ulcer "+ve control".

3- DPY- treated group. DPY was suspended in 2% Arabic gum solution and orally administered at a dose of 10 mg/kg once daily in a volume of 5 ml\kg for 2 weeks. The last dose was administered 30 min before CRS induction.[12]

After completion of the 3 hours of stress, rats were killed by using an overdose of ether. Their stomachs were removed, opened along the greater curvature and the gastric content of each stomach was collected.

ASSESSMENT OF GASTRIC MUCOSAL LESIONS

The stomachs were washed with ice-cold saline and scored for gross mucosal lesions, gastric mucosal lesions were examined using a magnifier and were given scores according to the method described by Biswas and his coworkers.[13]

MEASUREMENT OF MDA, GSH, NO, PGE2 AND MPO IN GASTRIC MUCOSA

The mucosa was scratched carefully, weighed and placed in a glass homogenizer containing 2 ml phosphate buffer (pH 7.4). After homogenization, by using motor-driven Teflon pestle, the homogenate was immediately stored at -80^{o} C. Assessment of gastric mucosal content of MDA[14], NO[15], (GSH) levels[16] and MPO activity[17] were performed by spectrophotometer. PGE_2 level was determined by enzyme-linked immunosorbent assay (ELISA) using PGE_2 immunoassay kit.

DETERMINATION OF MUCIN CONCENTRATION IN GASTRIC JUICE

The collected gastric juice after opening the stomach was centrifuged for 15 minutes at 3000 rpm to remove any solid debris and the supernatant was used for determination of mucin concentration. The used method is based on the determination of the hexose component of the mucin spectrophotometrically.[18]

HISTOPATHOLOGICAL EXAMINATION OF GASTRIC TISSUE

A reprehensive part of the stomach of each animal is used for histopathological examination. Gastric tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin for histological examination using light microscopy.

STATISTICAL ANALYSIS

Data were analyzed by using GraphPad Prism® (Version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com) and expressed as mean ± standard error of mean (SEM). One way Analysis of Variance (ANOVA) was done followed by post hoc Dunnet's test. A probability value less than 0.05 was considered statistically significant.

RESULTS

EFFECT OF DIPYRIDAMOLE ON ULCER INDEX IN CRS-INDUCED GASTRIC ULCER IN ADULT AND AGED RATS.

Induction of gastric ulcer by CRS methods in rats leads to a significant increase in the U.I. in both CRS adult and aged rats compared to normal non CRS adult and aged rats, respectively. The ulcer index (U.I.) in aged rat groups was more significantly increase compared to adult rat groups (table 1). DPY treated both adult and aged rats showed a significant decrease in this elevated U.I. (table 1).

Table: 1 Effect of dipyridamole on ulcer index and gastric ulcer in CRS-induced gastric ulcer in adult and aged rats.

Measured Parameter / Animal groups	Adult U.I.	Aged U.I.
Normal control (-ve control)	0	0
CRS - induced (+ve control)	45.40 ± 2.86 *	48.15 ± 3.82 *○
DPY- treated group	25.87 ± 1.08●	28.10 ± 1.54●○

Data are mean ± SEM of 8 rats.
*Significantly different from normal control rats (–ve control) at $p < 0.05$.
●Significantly different from CRS rats (+ve control) at $p < 0.05$.
○ Significantly different aged vs adults rats at $p < 0.05$.

EFFECT OF DIPYRIDAMOLE ON THE GASTRIC MUCOSAL CONTENT OF MDA IN CRS-INDUCED GASTRIC ULCER IN ADULT AND AGED RATS.

A significant increase was obtained in the gastric mucosal content of MDA in both CRS adult and aged rats compared to normal non CRS adult and aged rats, respectively. A significantly increase was observed in the gastric mucosal content of MDA in aged rats compared to adult rats (table 2).

A significant decrease was observed in the gastric mucosal content of MDA in both CRS adult and aged rats treated by DPY compared to CRS adult and aged rats, respectively (table 2).

EFFECT OF DIPYRIDAMOLE ON THE GASTRIC MUCOSAL CONTENT OF GSH IN CRS-INDUCED GASTRIC ULCER IN ADULT AND AGED RATS.

A significant decrease was obtained in the gastric mucosal content of GSH in both CRS adult and aged rats compared to normal non CRS adult and aged rats, respectively. A significantly decrease was observed in the gastric mucosal content of GSH in aged rats compared to adult rats (table 2).

A significant increase was observed in the gastric mucosal content of GSH in both CRS adult and aged rats treated by DPY compared to CRS adult and aged rats, respectively (table 2).

EFFECT OF DIPYRIDAMOLE ON GASTRIC MUCOSAL CONTENT OF NO IN CRS-INDUCED GASTRIC ULCER IN ADULT AND AGED RATS.

A significant decrease was obtained in the gastric mucosal content of NO in both CRS adult and aged rats compared to normal non CRS adult and aged rats, respectively. A significantly decrease was observed in the gastric mucosal content of NO in aged rat groups compared to adult rat groups (table 2).

A significant increase was observed in the gastric mucosal content of NO in both CRS adult and aged rats treated by DPY compared to CRS adult and aged rats, respectively (table 2).

Table (2): Effect of dipyridamole on gastric mucosal Content of MDA, GSH and NO in CRS-induced gastric ulcer in adult and aged rats.

Measured Parameter / Animal groups	Adult			Aged		
	MDA (nmol/gm wet tissue)	GSH (µmol/gm wet tissue)	NO (nmol/gm wet tissue)	MDA (nmol/gm wet tissue)	GSH (µmol/gm wet tissue)	NO (nmol/gm wet tissue)
Normal control (-ve control)	20.56 ± 1.70	15.05 ± 0.7	161.4 ± 3.8	25.39 ± 1.2○	12.13 ± 0.9○	149.1 ± 3.7○
CRS - induced (+ve control)	58.31 ± 2.43 *	10.35 ± 0.5 *	113.4 ± 5.5 *	69.15 ± 2.6 *○	8.63 ± 0.4 *○	90.20 ± 7.1 *○
DPY - treated group	37.30 ± 2.40 ●	12.57 ± 0.5 ●	140.4 ± 7.3 ●	45.72 ± 2.5 ●○	11.10 ± 0.5 ●○	115.0 ± 5.8 ●○

Data are mean ± SEM of 8 rats.
*Significantly different from normal control rats (–ve control) at $p < 0.05$.
●Significantly different from CRS rats (+ve control) at $p < 0.05$.
○ Significantly different aged vs adults rats at $p < 0.05$.

EFFECT OF DIPYRIDAMOLE ON GASTRIC MUCOSAL CONTENT OF PGE2 IN CRS-INDUCED GASTRIC ULCER IN ADULT AND AGED RATS.

A significant decrease was obtained in the gastric mucosal content of PGE2 in both CRS adult and aged rats compared to normal non CRS adult and aged rats, respectively. A significant decrease was observed in the gastric mucosal content of PGE_2 in aged rat groups compared adult rat groups (figure 1).

A significant increase was observed in the gastric mucosal content of PGE2 in both CRS adult and aged rats treated by DPY compared to CRS adult and aged rats, respectively (figure 1).

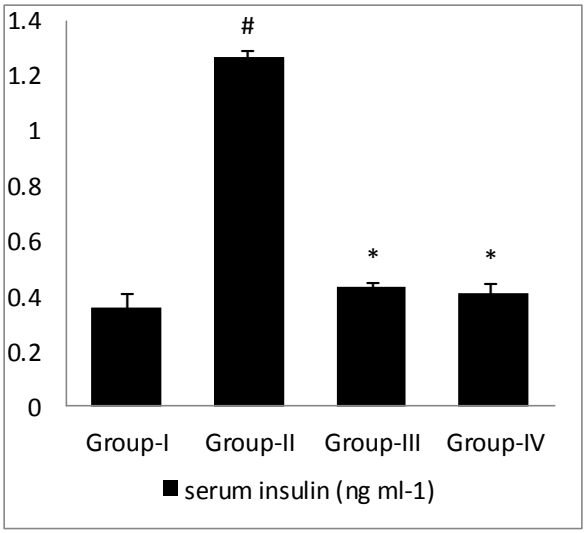

Figure (1): Effect of dipyridamole on gastric mucosal content of PGE2 in CRS-induced gastric ulcer in adult and aged rats.

Data are mean ± SEM of 8 rats.
*Significantly different from normal control rats (–ve control) at $p < 0.05$.
•Significantly different from CRS rats (+ve control) at $p < 0.05$.
º Significantly different aged vs adults rats at $p < 0.05$.

EFFECT OF DIPYRIDAMOLE ON GASTRIC JUICE MUCIN CONTENT IN CRS-INDUCED GASTRIC ULCER IN ADULT AND AGED RATS.

A significant decrease was obtained in the gastric juice mucin content in both CRS adult and aged rats compared to normal non CRS adult and aged rats, respectively. A significantly decrease was observed in the gastric mucosal content of mucin in aged rat groups compared to adult rat groups (figure 2).

A significant increase was observed in the gastric juice mucin content in CRS adult and aged rats treated by DPY compared to CRS adult and aged rats (Figure 2).

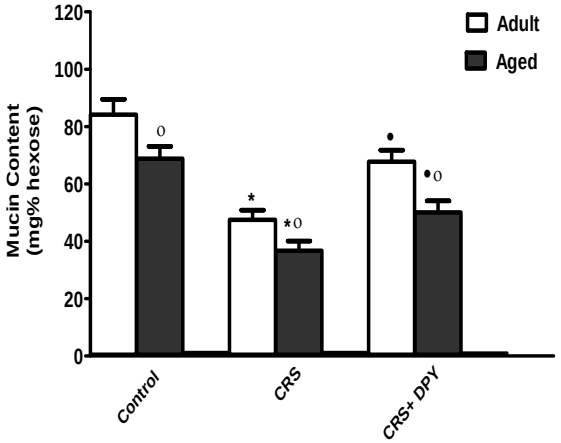

Figure (2): The Effect of DPY on Gastric Juice Mucin Content in Adult and Aged Rats.

Data are mean ± SEM of 8 rats.
*Significantly different from normal control rats (–ve control) at $p < 0.05$.
•Significantly different from CRS rats (+ve control) at $p < 0.05$.
º Significantly different aged vs adults rats at $p < 0.05$.

THE EFFECT OF DIPYRIDAMOLE ON GASTRIC MUCOSAL MPO ACTIVITY IN CRS-INDUCED GASTRIC ULCER IN ADULT AND AGED RATS.

A significant increase was observed in the gastric mucosal MPO activity in both CRS adult and aged rats treated by DPY compared to CRS adult and aged rats, respectively. A significant increase was observed in aged rat groups compared to adult rat groups (figure 3). A significantly decrease was obtained in the gastric mucosal MPO activity in DPY treated in both adults and aged rats compared to both adult and aged CRS control, respectively (figure 3).

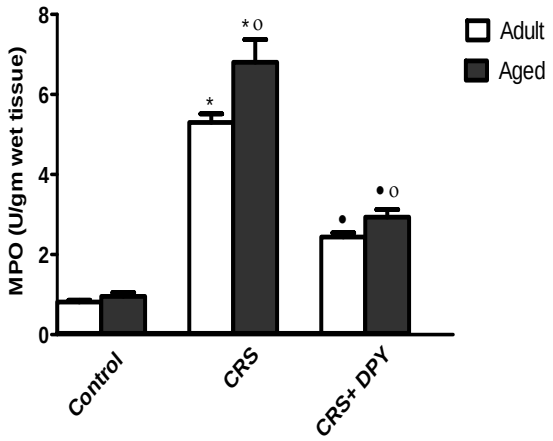

Figure (3): The Effect of Dipyrdamole on CRS on Gastric Mucosal MPO activity in Adult and Aged Rats.

Data are mean ± SEM of 8 rats.
*Significantly different from normal control rats (–ve control) at $p < 0.05$.
•Significantly different from CRS rats (+ve control) at $p < 0.05$.
º Significantly different aged vs adults rats at $p < 0.05$.

HISTOPATHOLOGICAL EXAMINATION RESULTS

Samples of the gastric wall from all groups were obtained for histopathological examination and the results were reported as follow:

1: ADULT RATS

The gastric mucosa of the -ve control rats (Figure 4A) showed no mucosal ulceration.
The gastric mucosa of the CRS - induced rats (+ve control) showed large deep wide mucosal ulcer (Figure 4B).
The gastric mucosa of the DPY- treated group (group 6) (Figure 4C) showed small shallow mucosal ulcer with exfoliated cells.

2: AGED RATS

The gastric mucosa of the -ve control rats (Figure 5A) showed no mucosal ulceration.

The gastric mucosa of CRS (+ve control) rats showed deep ulcer (Figure 5B).

The gastric mucosa of the DPY treated group in aged rats (Figure 5C) showed small mucosal ulcers.

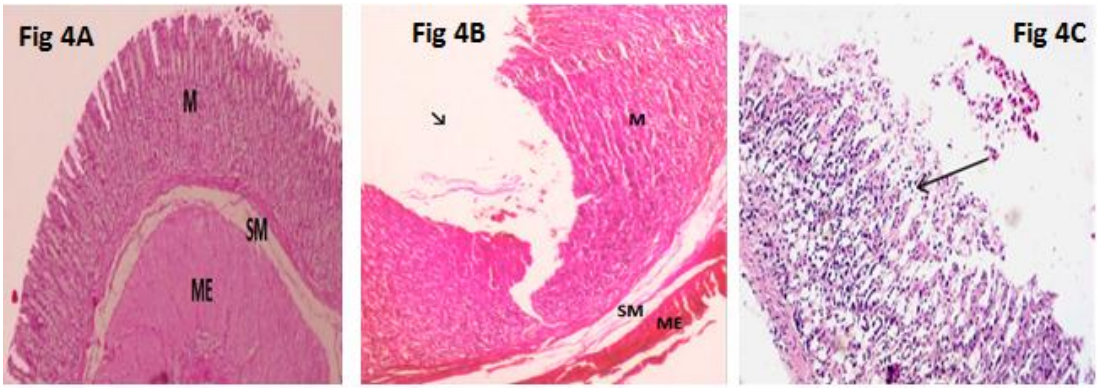

Figure 4A: Microscopic examination of gastric wall of control adult rat showing normal gastric.
Figure 4B: Microscopic examination of gastric wall of CRS adult rat showing large deep wide gastric ulcer (arrow).
Figure 4C: Microscopic examination of the gastric wall of CRS adult rat received DPY showing small shallow mucosal ulcer with exfoliated cells (arrow).
Note; mucosa (M), sumucosa (SM) and muscularis externa (ME)

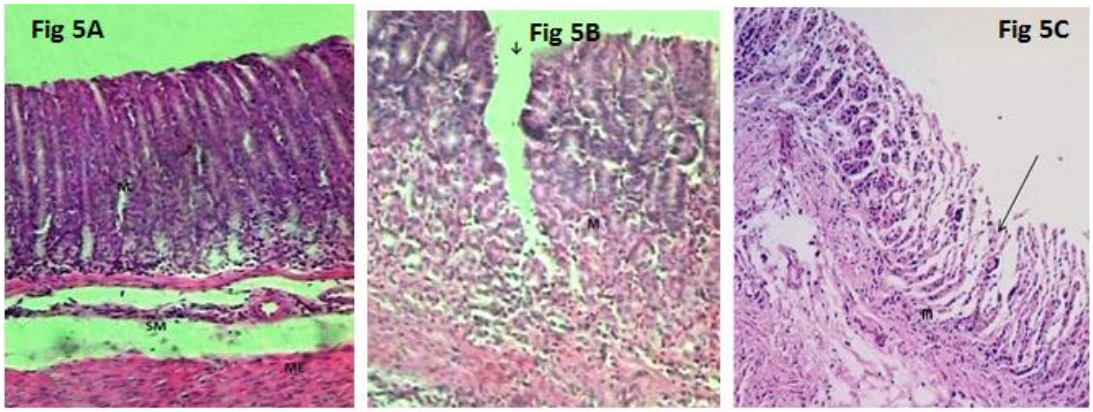

Figure 5A: Examination of the gastric wall of an aged negative control rat showing normal mucosa.
Figure 5B: Examination of the gastric wall of CRS aged rat showing deep ulcer (arrow) in the mucosa.
Figure 5C: Examination of the gastric wall of a normal aged rat received DPY showing shallow ulcer (arrow) in the mucosa.
Note; mucosa (M), sumucosa (SM) and muscularis externa (ME)

DISCUSSION

Due to the increase in the aged population in many countries, physicians have recognized that the clinical profile of diseases may differ between young and aged people. Gastric ulcers are a common disease and an increase in the incidence of gastric ulcers was observed in aged persons, in addition, refractory ulcers are often encountered in the aged population.[19]

Stress induces acute gastric mucosal lesions by complex psychological factors lead to decrease blood flow to the mucosa, increase in muscular contractility, leukocyte activation and increased free radical generation.[20] Stress is also believed to cause mucosal ischemia, leading to increased generation of ROS and lipid peroxidation.[21] Such increased activity of ROS often leads to mucosal damage with the subsequent destruction of epithelial basement membrane functioning.[22]

CRS-induced ulceration is known to occur due to the influence of both physiological and psychological factors.[23] The development of the gastric mucosal lesions in CRS could be attributed to increased gastric motility, vagal overactivity, mast cell degranulation, and decreased gastric mucosal blood flow. In addition, CRS can possibly lead to decrease PG synthesis.[24]

The results obtained in the present study clearly showed that rats exposed to CRS developed gastric lesions as indicated by a significant increase in ulcer index. The ulcer index was higher–significantly higher in aged rats compared to adult rats. This suggests that the aged rats

are more susceptible to peptic ulcer than adult rats. The results of this study are in accordance with the previous studies which indicated that aged gastric mucosa has impaired mucosal defense mechanisms such as decreased mucus and bicarbonate secretion, decreased PG production, and reduced NO synthase activity, reduction in mucosal blood flow and, impaired sensory nerve responses to acid.[25,26] Besides, Experimental and clinical studies indicate that the aging gastric mucosa has prominent structural and functional changes compared to young gastric mucosa that impair gastric mucosal defense.[27,28,29] Moreover, aged gastric mucosa has increased susceptibility to injury by a variety of damaging agents such NSAIDs, ethanol, and hypertonic solution.[30]

The current study demonstrated DPY possesses a significant reduction in the ulcer index in both adult and aged rats compared to their control groups.

Oxidative stress is associated with increased production of ROS with a significant decrease in antioxidant defense capability.[31] Lipid peroxidation causes loss of membrane fluidity, impaired ion transport and membrane integrity, and finally loss of cellular functions.[32] Antioxidant enzymes as GSH contribute to the gastric oxidative-antioxidative balance. GSH has an important free radicals scavenger effect.[33]

In the present study, gastric mucosal content of MDA was significantly increased while the level of GSH was significantly decreased in CRS control in both adult and aged rats and these changes is more significant in aged rat groups compared to adults rat groups. These results indicate that gastric mucosa of aged rats is susceptible to oxidative stress than adult rats. This result is in agreement with the previous reported studies.[2,30,34]

DPY was proven to exhibit antioxidant activity in this study. It significantly decreased the rise of gastric mucosal MDA level and augmented the decrease of GSH level in both adult and aged CRS rats. These results are in harmony with the previously reported studies where it use in several oxidative stress associated diseases; including atherosclerosis, thrombosis, CNS diseases, and cancer.[35] It inhibits lipid peroxidation and ameliorates oxidative stress in other previous studies.[36,37]

Regarding, NO which is a double-edged weapon exerting either protective or destructive effects depending on the extent of NO synthesis.[38] NO generated from constitutive nitric oxide synthase (NOS) plays an important role in gastric ulcer healing[39], and beneficial in maintaining the mucosal integrity[40], whereas NO generated from inducible NOS participates in ulcer formation through the production of ROS and their cytotoxic actions.[41] In addition, prostaglandins especially PGE_2, plays important roles in maintaining the integrity of the gastrointestinal mucosa, including stimulation of mucus and bicarbonate secretion, resistance of epithelial cells to injury, and decrease the release of inflammatory mediators. NO plays a key role in the maintenance of the gastrointestinal mucosa and that both NO and prostaglandins share similarities in various gastrointestinal functions. NO and prostaglandin mediated mechanisms of mucosal defense also exhibit a degree of cooperation.[42,43]

In the present study, both adult and aged CRS-induced rats were associated with significant decrease in gastric mucosal content of NO and PGE_2 and gastric juice content of mucin. This decrease was more significant in aged rats compared to adult rats. These results are in accordance with other experimental and clinical studies indicate that aging gastric mucosa has impaired mucosal defenses such as decreased mucus and bicarbonate secretion, decreased prostaglandin generation, reduced NOS activity and reduced blood flow.[26,28] In a previous study, water immersion stress reduced both gastric mucosal PG content and NOS activity in young rats and the reduction of cytoprotective effects based on PG seems to be controlled easily compared with NO. Moreover, NO production was reported to decrease with ageing.[19]

In this study, both adult and aged DPY treated groups showed significant increase in gastric mucosal content of NO and PGE_2, and gastric juice content of mucin. The results obtained in the present study are in inconsistent with previously reported results that indicated DPY enhanced NO action via inhibition of cGMP degradation by phosphodiesterase and also increased eNOS expression via cAMP-dependent protein kinase pathway.[36] Thus, It could postulated that the protective effect of DPY against CRS-induced gastric is due to increasing NO leading to maintenance of mucosal blood flow and increased PGE_2 production with subsequent increase in gastric juice content of mucin.

MPO enzyme is widely used as an index of neutrophil infiltration in various gastric injuries that has been previously described to be a key contributor in chronic inflammatory diseases.[44]

A significant increase in gastric mucosal MPO activity was observed in both adult and aged CRS-induced gastric ulcer in this study. These results are in agreement with the previous published data.[45.46] This increase was more obvious in aged rats compared to adult rats. These results indicate that the gastric mucosa in aged rats is more susceptible for inflammatory process than adult rats. Another study demonstrated that chronic inflammation is considered as a major risk factor underlying aging and age-related diseases.[47]

Biochemically, the anti-inflammatory effect of DPY was confirmed by a significant decrease in gastric mucosal MPO activity in both adult and aged rats. These results are in agreement with Marques and his colleges[48] who reported that DPY decreased MPO activity in implants

placed in peritoneal cavity of mice. Other studies have reported the anti-inflammatory effect of DPY.[49,50]

Lastly, the histopathological study of DPY-treated in adults and aged rats indicates status of structural integrity of the gastric mucosa and provides further support to the protective effect of DPY in CRS induced gastric ulcer in rats.

CONCLUSION

Taken together, the current study demonstrates a possible gastroprotective effect of DPY against CRS-induced gastric ulcer in both adult and aged rats. The protective effect of DPY is mediated, in part, via anti-oxidant and anti-inflammatory properties alongside enhancement of the gastric mucosal barrier.

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