

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

السَّلَامُ عَلَيْكُمْ وَرَحْمَةُ اللَّهِ وَبَرَكَاتُهُ



Agmatine Protects Against Gastric Ischemia-Reperfusion Injury Mediated by AKT/IPK3

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Introduction



Agmatine

- ❑ An amine recently identified as an endogenous clonidine-displacing substance in mammalian brain.
- ❑ Generated by decarboxylation of L-arginine by the enzyme arginine decarboxylase.
- ❑ A biologically active substance.
- ❑ Its mode & sites of action have not been defined.



Agmatine ... (Continued)

- ❑ It binds with high affinity to both α 2- adrenoceptors of all subclasses & to the imidazoline receptors of the I1 & I2 subclasses.
- ❑ It is widely distributed in mammalian tissue, such as, brain, **stomach**, intestine & aorta.
- ❑ Thus agmatine may act as a neurotransmitter / modulator.



Agmatine ... (Continued)

- ❑ Recent studies showed that agmatine is protective against ischemia reperfusion injury in some organs including brain.
- ❑ However, no previous reports on its protective effect in gastric injuries:
 - ❑ On the contrary, it is deleterious in ethanol-induced gastric lesions.
 - ❑ Very recently (2009), it was reported that it is protective in human helicobacter pylori.



Objectives

The main objective of the present study was to investigate whether or not agmatine administration induces any gastric protective effect when given prior to gastric IR injury in the rat, & mechanisms involved.



Methods & Materials



Animals

- ❑ Male Wistar rats (weighing 170–210 g) were obtained from the College of Medicine Animal House at King Saud University (KSU).
- ❑ Rats were maintained on standard rat chow & tap water ad libitum.
- ❑ Rats were kept in an air-conditioned room with a 12 h day/light cycle.
- ❑ Animals were fasted 12 h prior to the experimental procedure.
- ❑ All studies were approved by the Ethics Committee of KSU.



Experimental design

- ❑ Rats were divided into three experimental groups (six rats/group):
 - (i) control sham-operated group;
 - (ii) I/R group; &
 - (iii) I/R + Agmatine group.

- ❑ Rats were anesthetized by intraperitoneal (IP) injection of urethane at a dose of 125 mg/100 g bodyweight (BW).



Experimental model

- ❑ The stomach was exposed, & the esophagus & the pylorus were occluded using bulldog clamps.
- ❑ The celiac artery was clamped & 100 mM HCL (1 mL/100 g BW) was placed in the stomach to maintain acid levels during ischemia.
- ❑ The acid was then removed 25 min after ischemia, & clamps were removed 30 min after ischemia.
- ❑ The tissues were allowed to reperfuse for 30 min, & then the stomach was removed & examined.

Experimental model

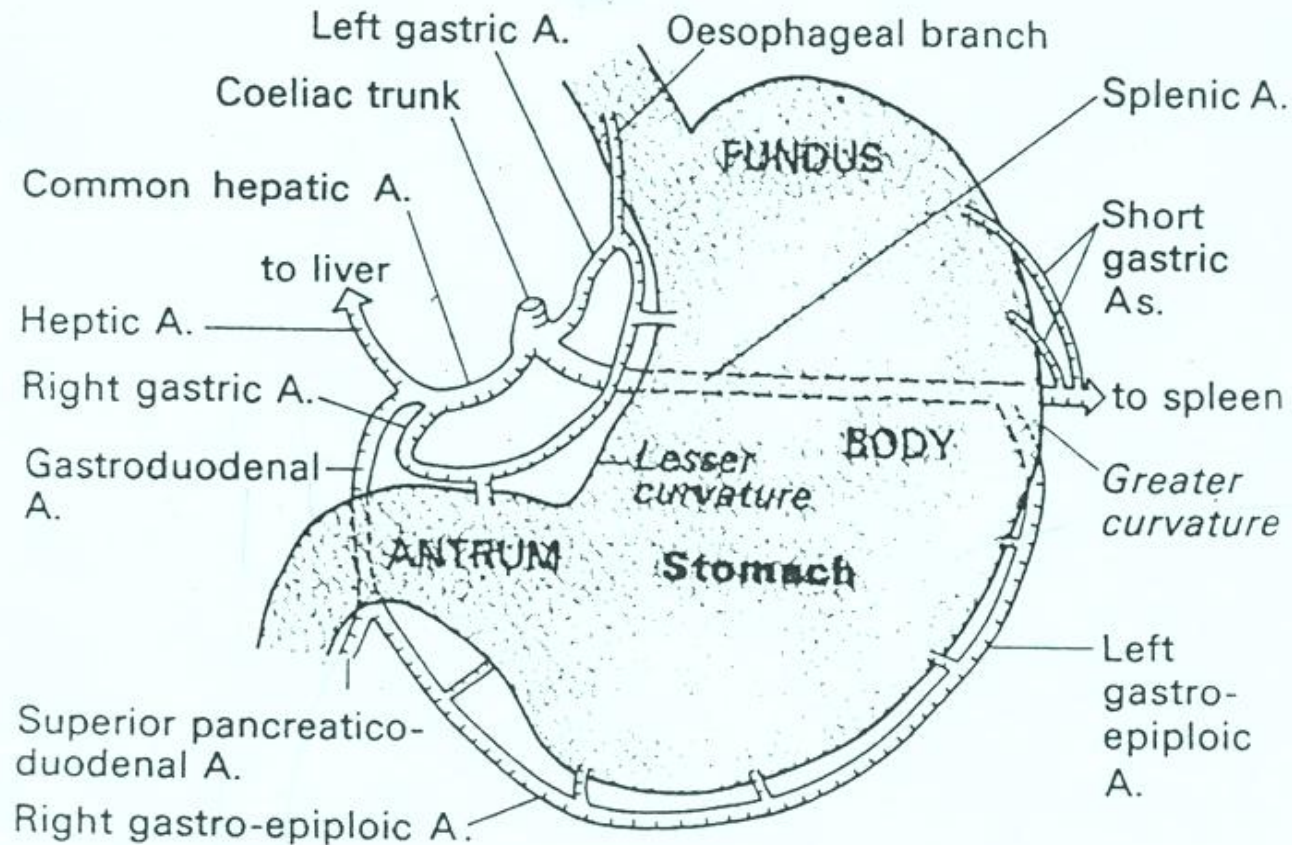


FIG. 11.19. Blood supply to stomach.

Methods



- ❑ For agmatine treated group, agmatine (100 mg/kg, i.p.) was administered 15 minues prior to induction of IR injury.
- ❑ By the end of the experiment, serum & gastric tissues were collected.
- ❑ Gastric samples were snap-frozen in liquid nitrogen & stored at -80°C for subsequent assays of vascular endothelial growth factor (VEGF).
- ❑ A piece of each stomach was fixed in 4% phosphate buffered formalin, embedded in paraffin & cut.

Methods



- ❑ **Gastric tissues were prepared for the following assessments:**
 1. Histological: stained with H& E.
 2. Immunostaining with sera specific for angiopoietin-1 & 2, (R&D Systems, USA).
 3. ELISA: VEGF



Determination of Vascular Permeability: Using Evan's Blue dye

- ❑ In another set including the 3 experimental groups, 1 mL of EB (0.5% v/w) was injected intravenously after reperfusion or sham operation.
- ❑ The amount of EB that accumulated in the stomach within the reperfusion period was measured:
 - Briefly, animals were killed & the stomach was removed.
 - Gastric content were collected carefully by lavaging with 5 mL of cold distilled water
 - The stomach was opened along the greater curvature & the corpus mucosa was scraped off & put into a tube containing 5 mL of distilled water.



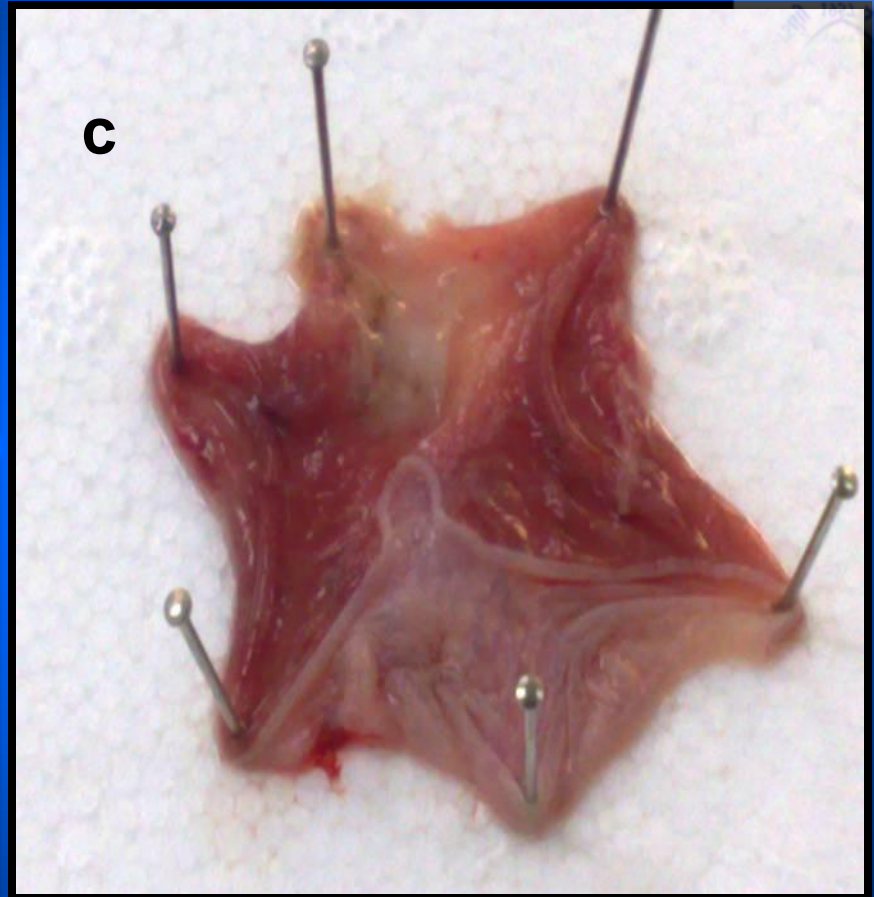
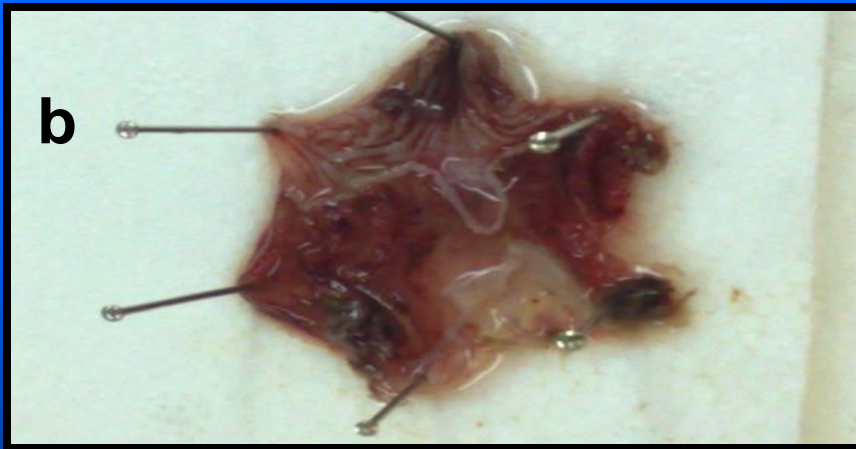
Determination of Vascular Permeability: Using Evan's Blue dye

- ❑ The EB was extracted by a modified method of Lange *et al.*, & its concentration was spectro-photometrically quantified:
 - The EB present in gastric contents & mucosa was extracted by adding 5 mL of formamide to each tube & kept in a shaking water bath at a temperature of 50°C for 24 h.
 - This was followed by centrifugation at 3000 g for 10 min & the absorbance of supernatant was measured at 612 nm (Lambada 5 Perkin-Elmer, Pomona, CA, USA).
 - The amount of EB was calculated from a previously made standard curve & expressed as micrograms per stomach.



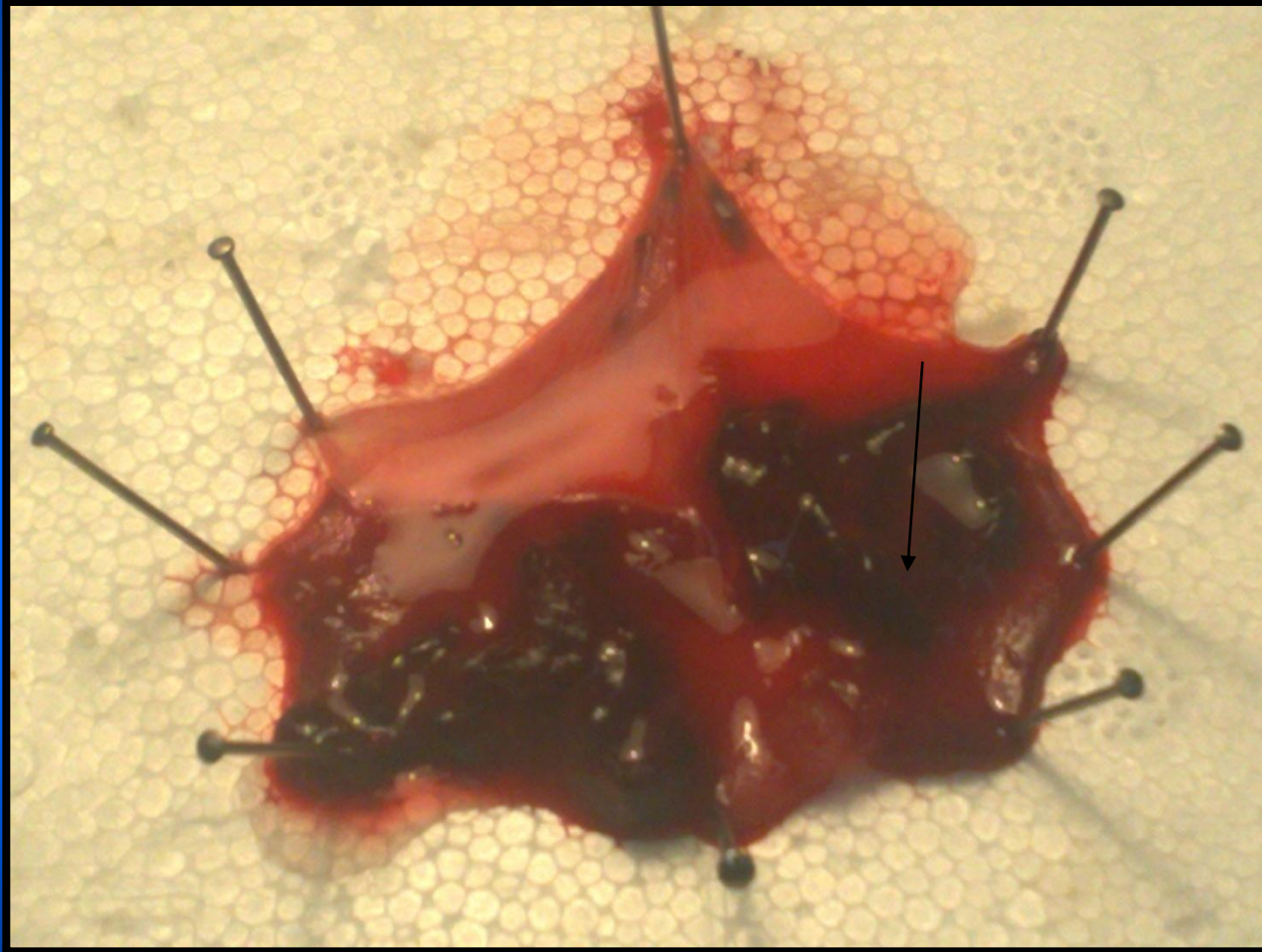
Results

Effect of agmatine pre-treatment on gastric ischemia reperfusion injury



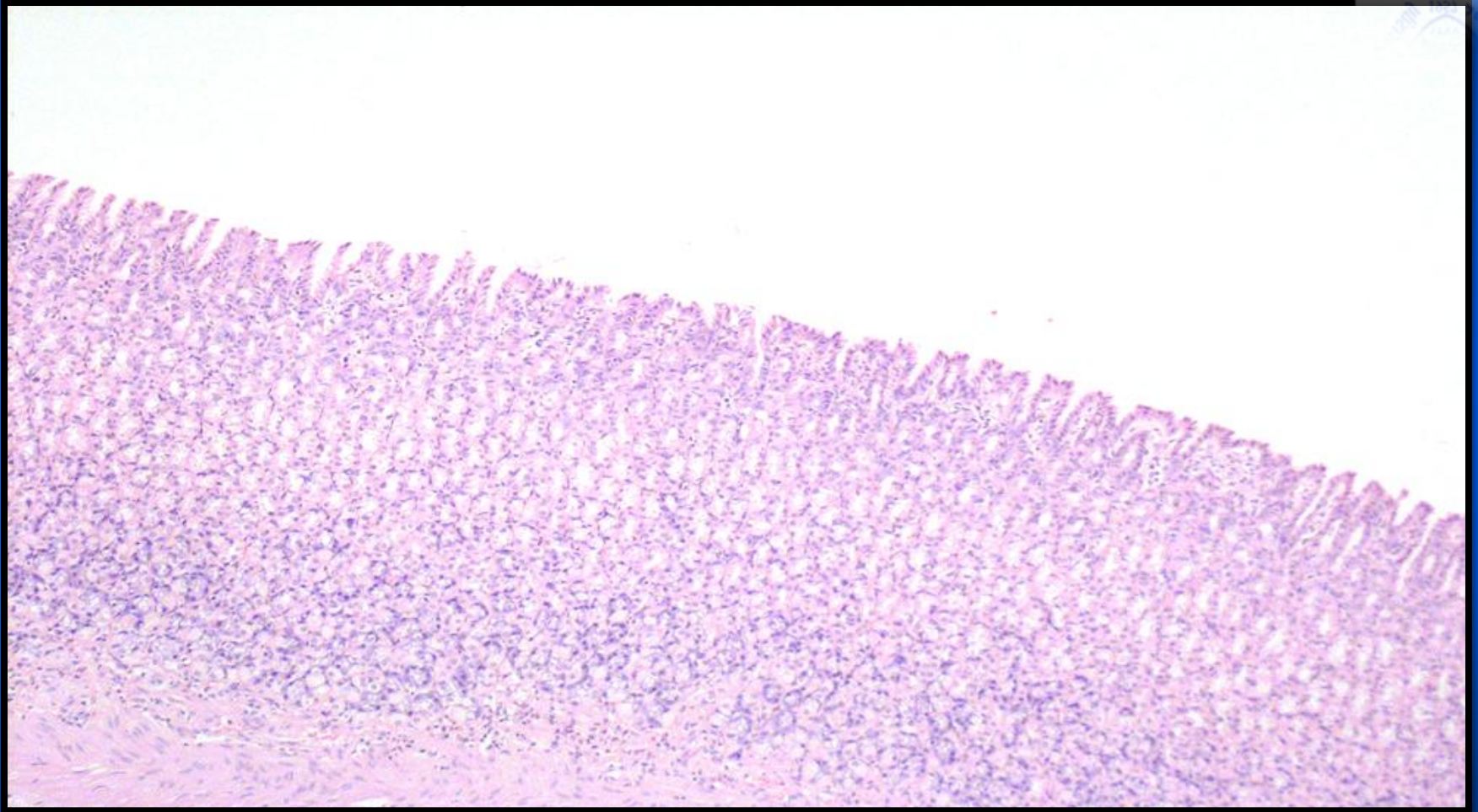
Normal gastric mucosa (a). Gross pathology of injured stomach (b) 30 minutes after reperfusion injury showing ulceration & extensive hemorrhage with blood clots. Agmatine prevented hemorrhage & reduced congestion (c).

Macroscopic changes in response to wortmanin (WM) administration prior to agmatine & IR treatment



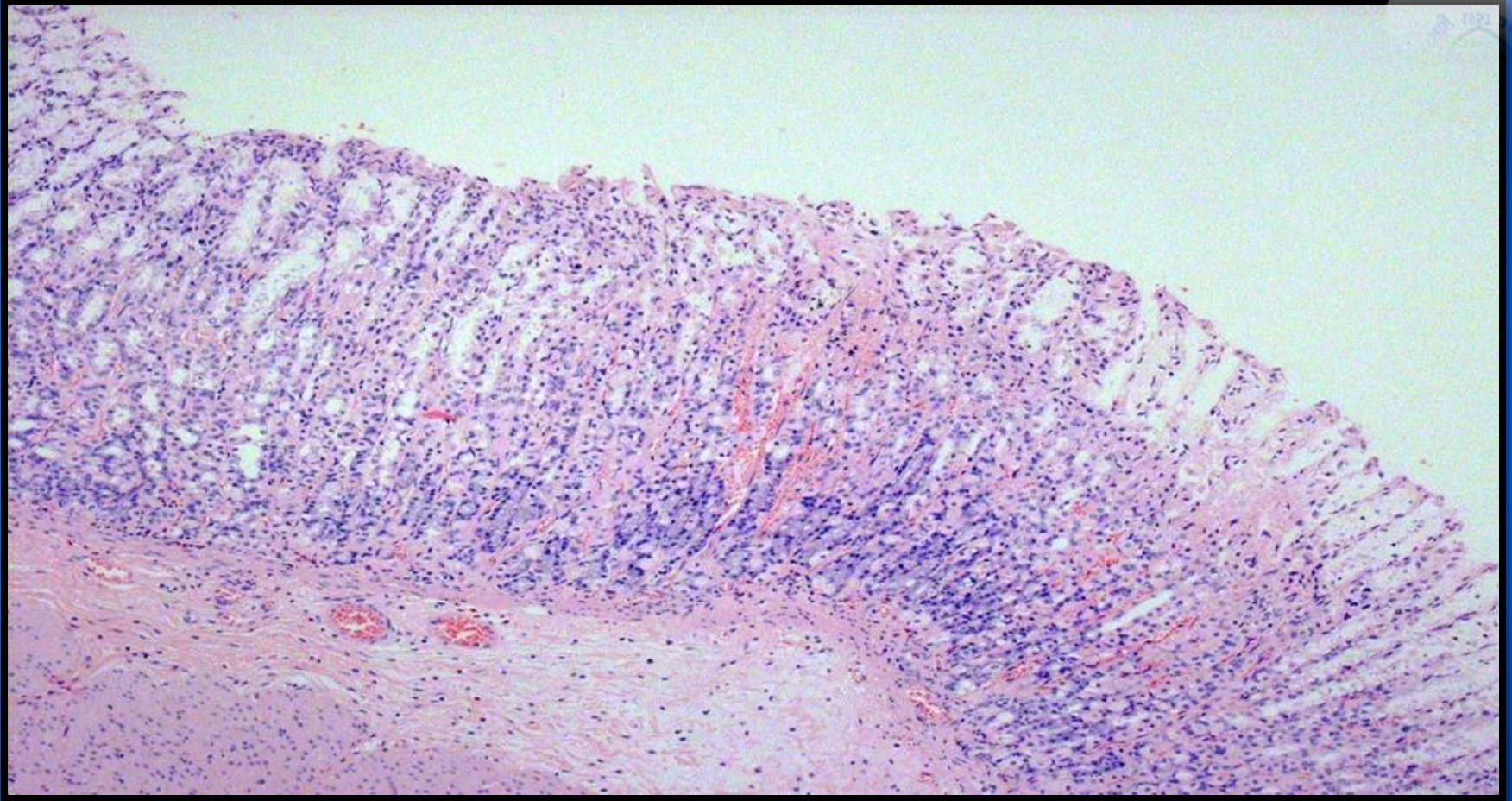
Effect of inhibition of AKt/IP3K by wortmanin on gastric protection induced by agmatine. Extensive hemorrhage & blood clots developed upon administration of wortmanin prior to agmatine treatment of rats exposed to gastric IR injury.

Normal gastric mucosa



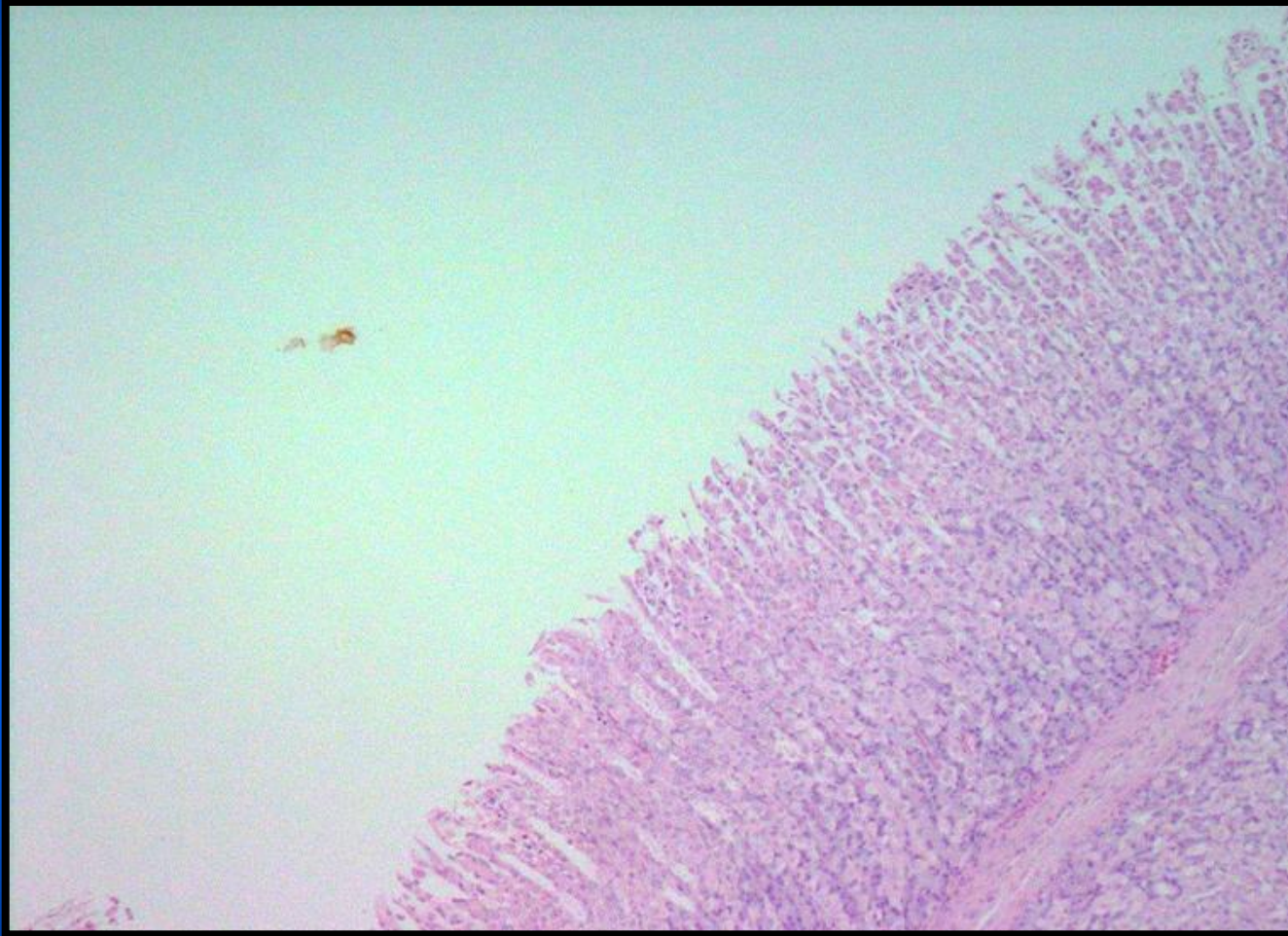
H&E stained paraffin embedded sections of normal gastric mucosa.
(Magnification X10).

Gastric IR



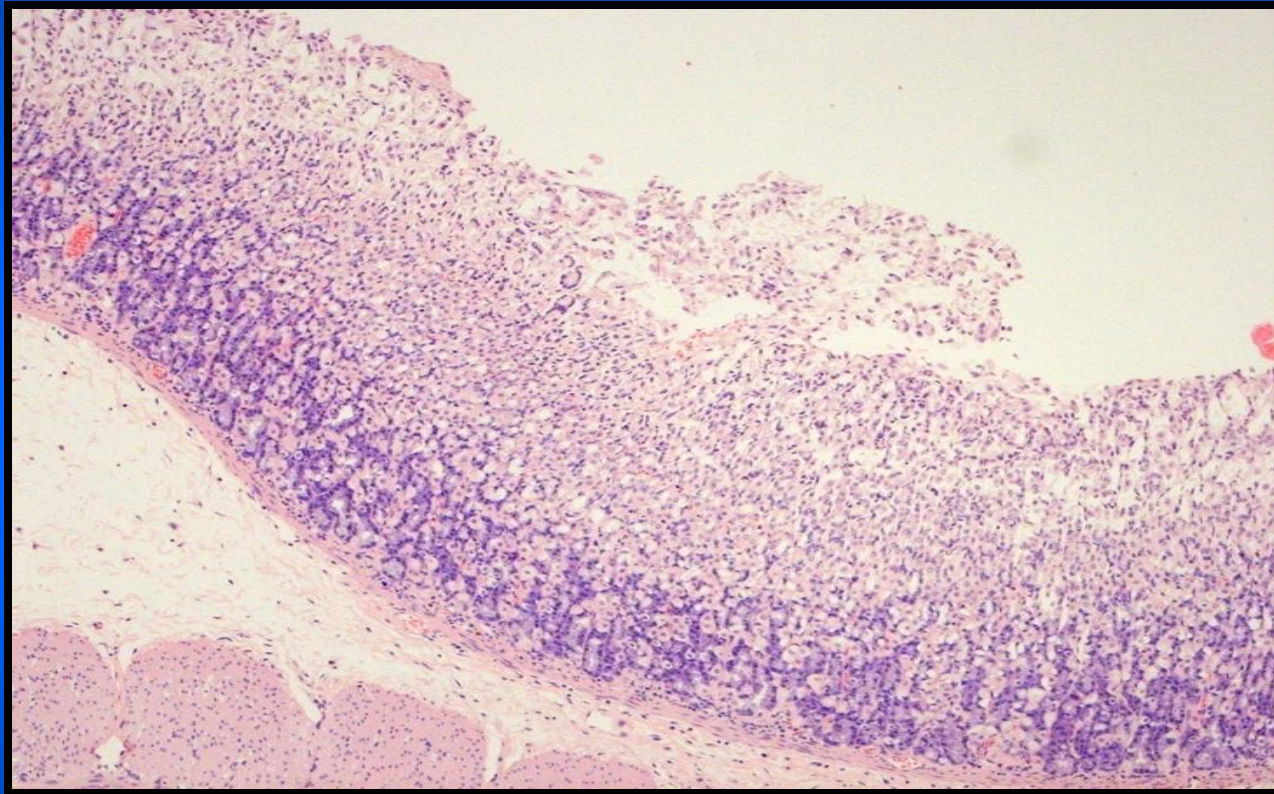
H&E stained paraffin embedded section of IR induced gastric injury. hemorrhage, ulceration & edema of gastric mucosa are shown. (Magnification X10).

Agmatine treatment



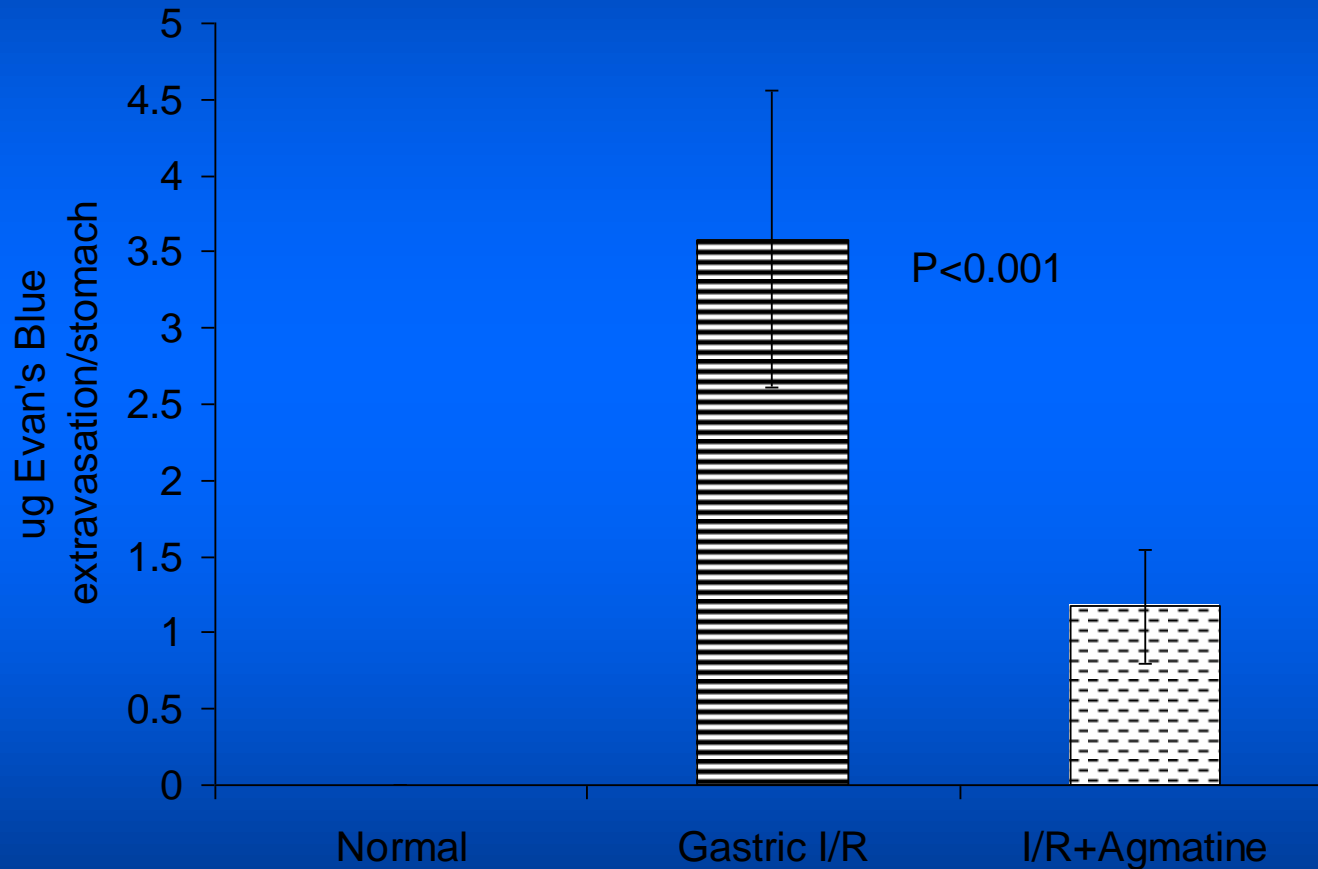
H&E stained paraffin embedded section of agmatine treated rats prior to IR induced gastric injury. Agmatine protected gastric mucosa from ischemic injury at a dose of 100 mg/kg. (Magnification X10).

Effect of Wortmanin administration prior to agmatine and IR treatment



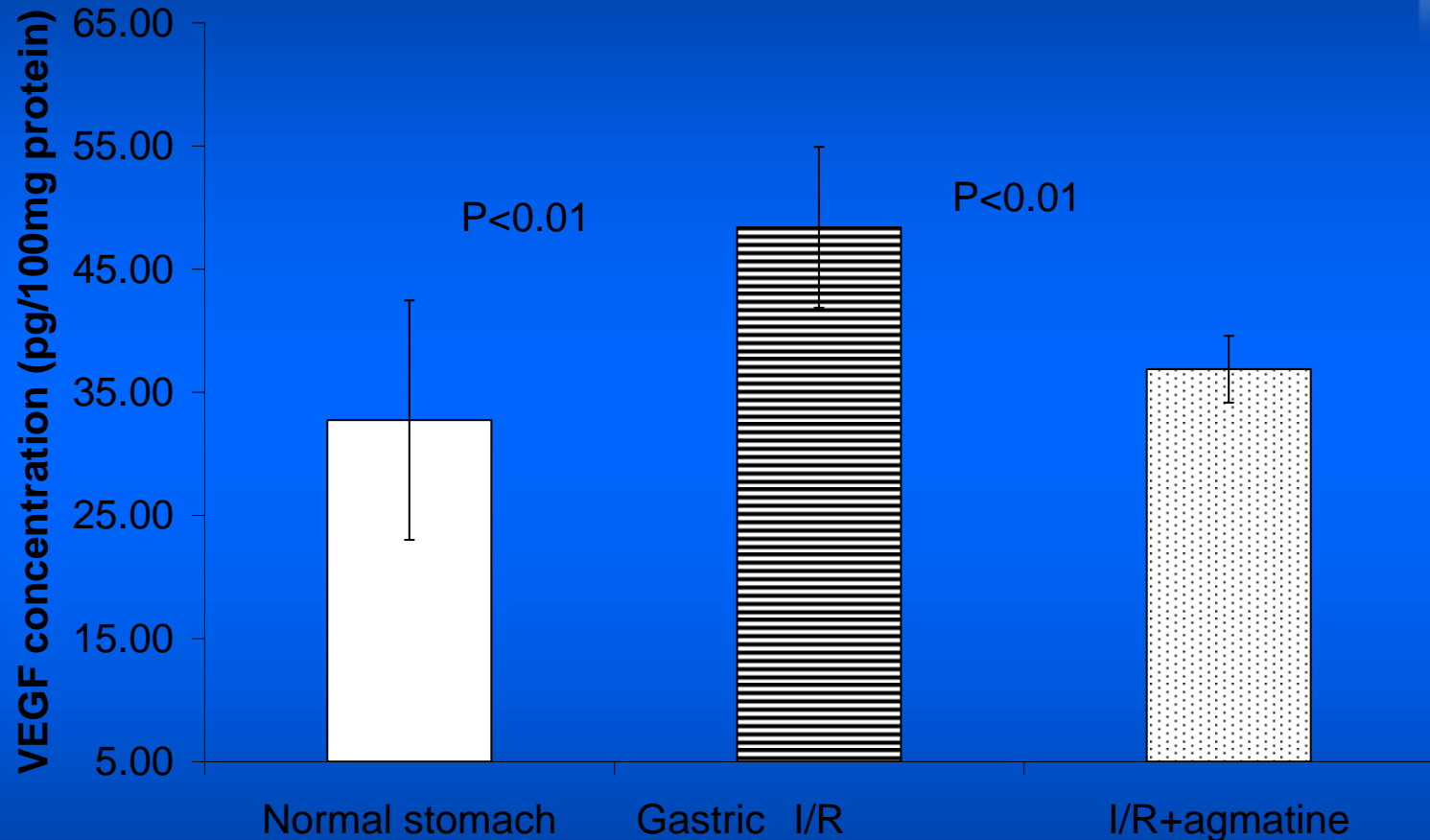
H&E stained paraffin embedded section of agmatine treated rats prior to IR induced gastric injury Wortmanin (WM) was administered prior to agmatine. The protection by agmatine was abolished upon treatment with WM an inhibitor of AKt/IP3K pathway. Extensive damage in form of ulceration, hemorrhage & edema are noticed. (Magnification X10).

Effect of agmatine on Evan's blue dye extravasation from stomach on exposure to reperfusion injury



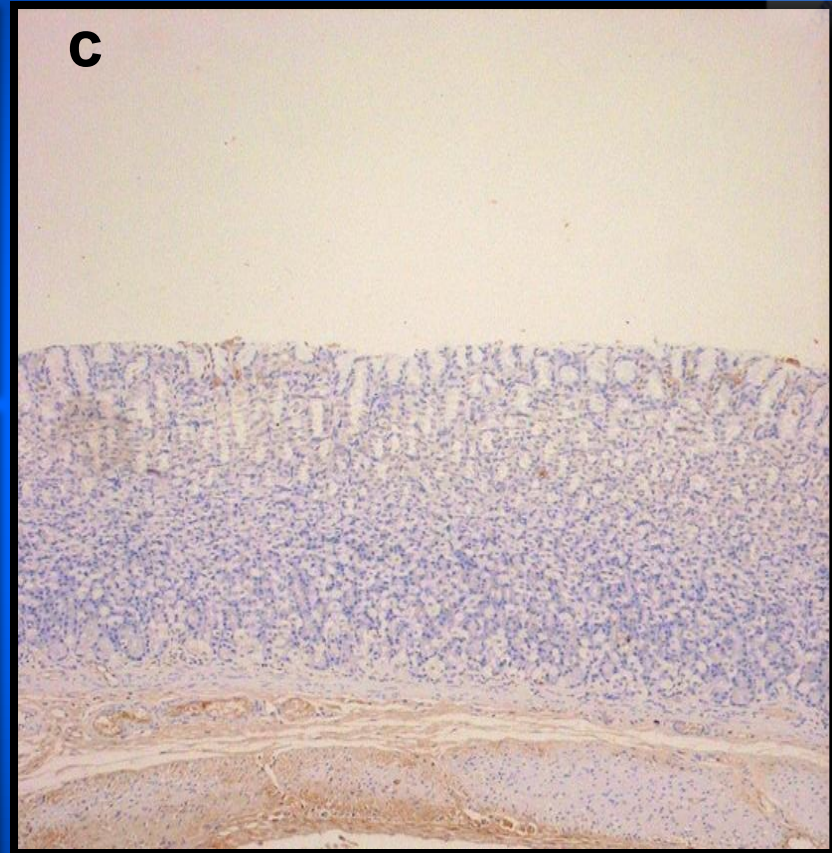
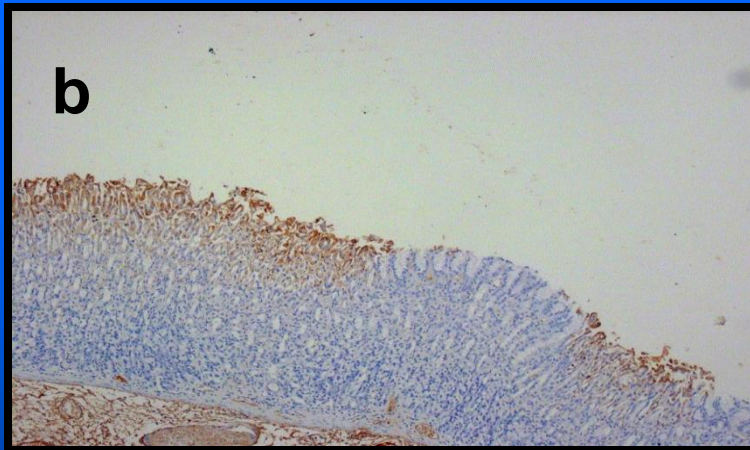
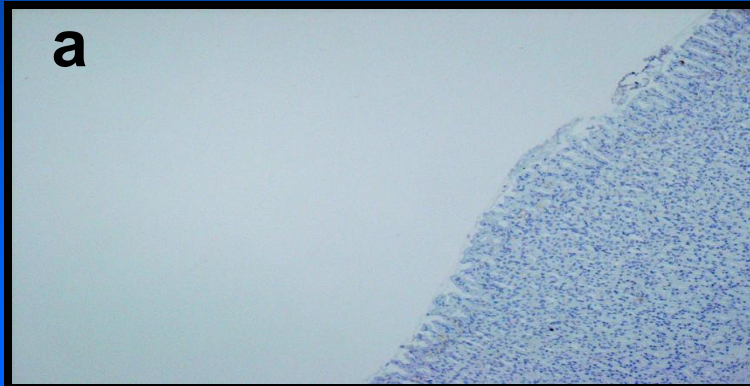
Ischemic gastric injury significantly induced extravasation of the dye. Agmatine administration attenuated the extravasation (ug/stomach) ($P<0.001$).

VEGF concentration in gastric tissue homogenate



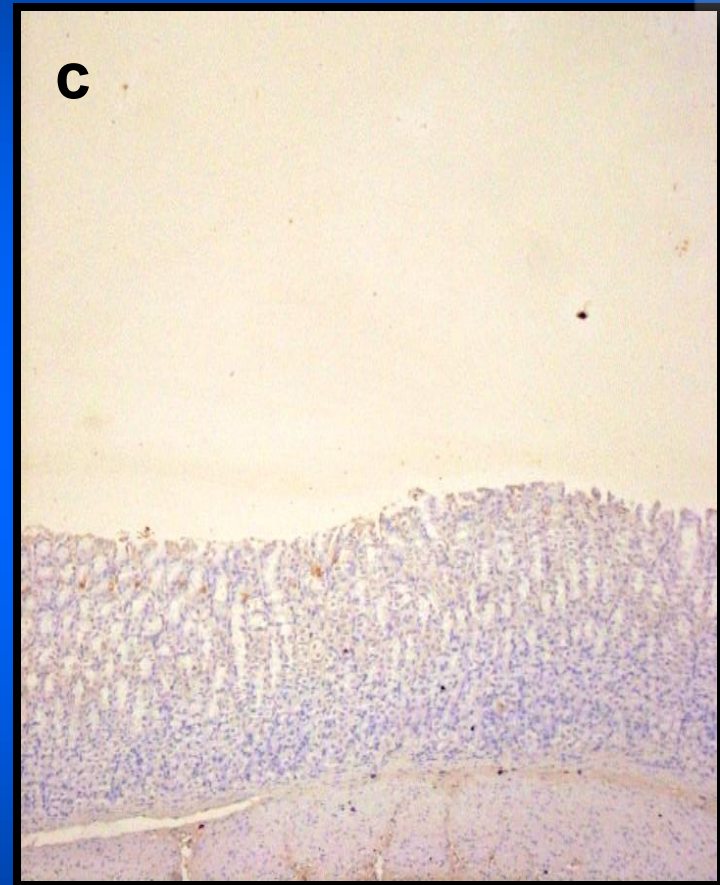
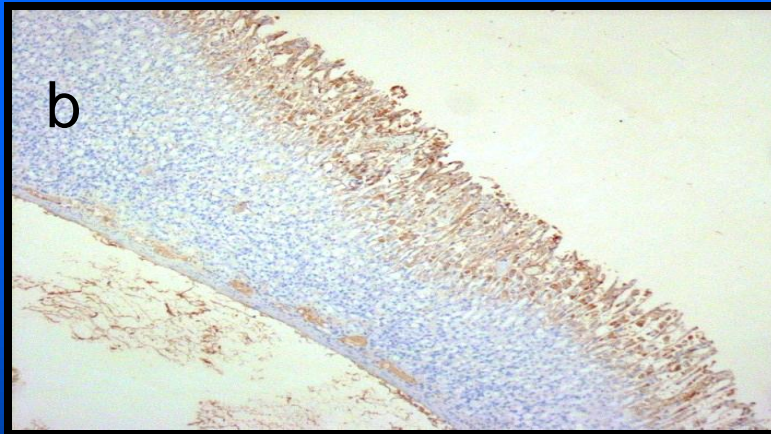
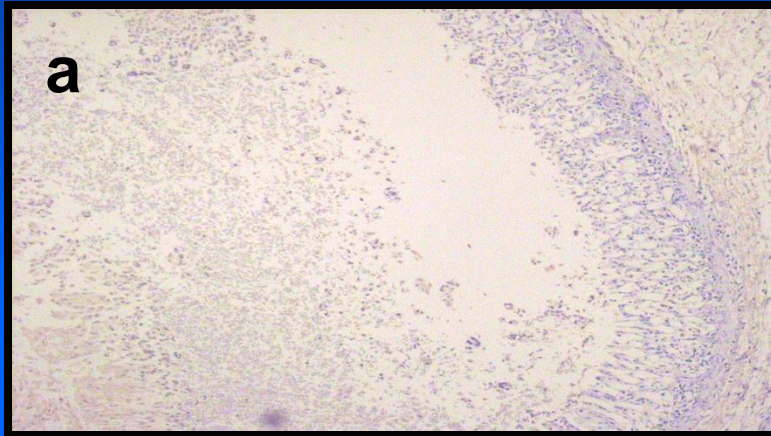
Agmatine pretreatment (100 mg/kg) reduced VEGF protein expression in gastric tissue with respect to IR injury.

Effect of IR injury and agmatine treatment on angiotensin-1 expression



Angiotensin-1 immunosaining. Seronegative staining for Angiotensin-1 of normal rat stomach **(a)**. Rat stomach subjected to I-R injury. Angiotensin is extensively expressed (brown) in areas where congestion & damage occurred, intact mucosa showed minimal expression **(b)**. Agmatine treated gastric sections. Agmatine pre-treatment (100 mg/kg) markedly attenuated the expression of angiotensin-1 **(c)**. (Magnification X10).

Effect of IR injury and agmatine treatment on angiotensin-2 expression



Seronegative staining for angiotensin-2 of normal rat stomach (a). Extensive expression of angiotensin-2 is seen mostly of the mucosa of rat stomach subjected to 30 minutes of ischemia & 60 minutes of reperfusion (b). Agmatine markedly attenuated angiotensin-2 expression and protected mucosa from injury when rat stomach treated with agmatine (100 mg/kg, i.p.) 15 minutes prior to induction of IR injury (30 minutes of ischemia & 60 minutes of reperfusion) (c). (Magnification X 10).

Conclusions

- ❑ Agmatine is protective in situations of gastric ischemic injury at least for brief periods of time.
- ❑ Agmatine induces gastric protection by reducing vascular permeability.
- ❑ The protective effect is probably involving AKt/IPK3 pathway.



Recommendations

- ❑ The study need to run over a longer time of exposure to ischemia to see whether it is protective as brief time or not.



Acknowledgment

- ❑ Research center – Center for Female Scientific & Medical Colleges.
- ❑ Animal House, College of Medicine, King Khalid University Hospital.
- ❑ King Saud University.

Thank You ...

