

ABSTRACT:

Acute liver injury (ALI) is a life-threatening syndrome accompanied by overwhelming apoptosis, necrosis and inflammation. The current study was directed to investigate and compare the potential hepatoprotective effect of bromelain (Brom) compared to silymarin (SLM) against D-galactosamine (GalN)-induced ALI in rats. SLM (100 mg kg⁻¹) and Brom (20 mg kg⁻¹ or 40 mg kg⁻¹) were orally administrated for 10 successive days. ALI was induced by a single dose of GalN (400 mg kg⁻¹i.p.) on the 8th day of the experiment. Blood samples were withdrawn on 10th day. Brom 20 group showed only significant amelioration of liver function tests (alanine and aspartate transaminases), inflammatory (tumor necrosis factor alpha and nuclear factor-kappa B) and apoptotic (caspase-8 and caspase-9) markers as well as histopathological alterations compared to GalN group. On the other hand, Brom 40 revealed further improvement and normalization of the previously mentioned markers. Interestingly, Brom 40 was much more effective than silymarin in ameliorating this type of liver injury. In conclusion, this study demonstrates a significant hepatoprotective effect of Brom against ALI induced by GalN.

Keywords: Acute liver injury; Bromelain; D-galactosamine; Silymarin

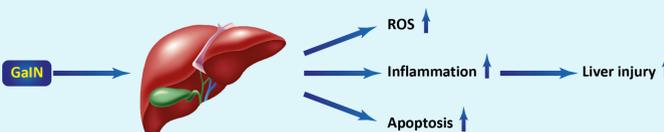
INTRODUCTION:

Acute liver injury (ALI) is a life-threatening syndrome accompanied by overwhelming apoptosis, necrosis, and inflammation⁽¹⁾.

D-galactosamine (GalN) is an experimental hepatotoxin amino sugar. It induces acute hepatotoxicity in rat liver via the production of reactive oxygen species (ROS) and depletion of UTP nucleotides⁽²⁾. This model is closely related to human viral hepatitis and acute self-limiting hepatitis with inflammation, necrosis, and regeneration, resembling a drug-induced liver disease in humans⁽³⁾.

Silymarin (SLM) is a standardized extract obtained from the seeds of milk thistle plant (*Silybum marianum*)⁽⁴⁾. It is used clinically for the treatment of liver diseases as a "hepatoprotective" agent⁽⁵⁾. It has free radicals scavenging ability and cytoplasmic stabilizing effect. Also, it has anti-inflammatory, immunomodulatory, and antifibrotic properties in the liver⁽⁶⁾.

Bromelain (Brom) is a crude pineapple extract. It contains thiol endopeptidases that have fibrinolytic, antithrombotic and anti-inflammatory activities⁽⁷⁾.



AIM OF THE WORK:

To investigate the possible hepatoprotective effect of Brom and compare it with SLM against GalN-induced ALI in rats.



MATERIAL & METHODS:

Adult male Albino rats were randomly allocated into five groups; each consisted of 8 rats.

Group 1: Received i.p injection of saline and served as a normal group.



Free access to food and tape water

Group 2: Received a single dose of GalN (400 mg/kg/i.p), in sterile saline on the 8th day of the experiment⁽⁸⁾.



Treatments were carried out for 10 successive days.

Group 3: SLM (100 mg/kg/p.o.)⁽⁹⁾

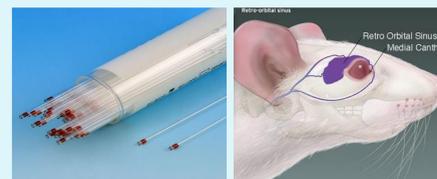
Group 4: Brom₂₀ (20mg/kg/p.o.)⁽¹⁰⁾

Group 5: Brom₄₀ (40mg/kg/p.o.)⁽¹⁰⁾

Groups (3,4,5) received a single dose of GalN solution in sterile saline on the 8th day of the experiment.



➤ Blood samples were withdrawn from the rats on the 10th day of the treatment period two hours after the last drug administration. Sera were isolated for aspartate transaminase (AST) and alanine transaminase (ALT) estimation.



➤ Parts of the liver were rinsed in chilled 0.9% NaCl, weighed, and homogenized. The homogenates were used for estimation of liver contents of:

- 1- Nuclear factor kappa B (NFκβ)
- 2- Tumor necrosis factor alpha (TNF-α)
- 3- Caspase 8 (Cas-8)
- 4- Caspase 9 (Cas-9)



RESULTS:

1- Liver Function Test:

Effects of SLM, Brom₂₀, and Brom₄₀ on AST and ALT activities in GalN-induced ALI in male rats.

Parameters	AST (U/ml)	ALT (U/ml)
Normal	15.00±1.24	12.67±1.17
GalN	59.33*±4.88	70.83*±6.20
SLM + GalN	40.33*±1.94	37.17*±1.45
Brom ₂₀ + GalN	47.33*±2.08	53.00*±1.48
Brom ₄₀ + GalN	18.50@±1.50	24.50@±0.85

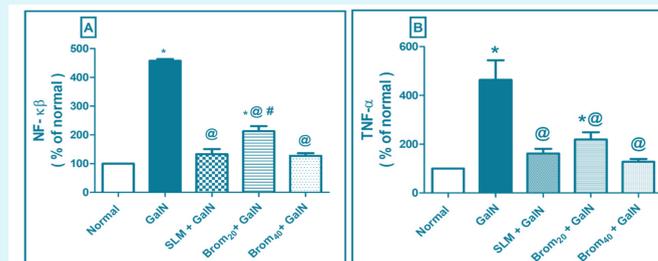
Each value represents the mean of 6 experiments ± S.E.M.

Statistical analysis was done using One-Way ANOVA followed by Tukey's post-hoc test.

*p < 0.05 vs. normal, @p < 0.05 vs. GalN, # p < 0.05 vs. SLM+ GalN

2- Inflammatory markers:

Effects of SLM, Brom₂₀, and Brom₄₀ on NF-κB (A) and TNF-α (B) contents in GalN-induced ALI in male rats.



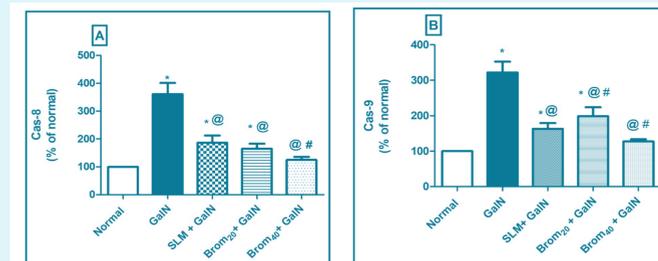
Each value represents the mean of 6 experiments ± S.E.M.

Statistical analysis was done using One-Way ANOVA followed by Tukey's post-hoc test.

*p < 0.05 vs. normal, @p < 0.05 vs. GalN, # p < 0.05 vs. SLM+ GalN

3- Apoptosis Markers:

Effects of SLM, Brom₂₀, and Brom₄₀ on Cas-8 (A) and Cas-9 (B) contents in GalN-induced ALI in male rats.



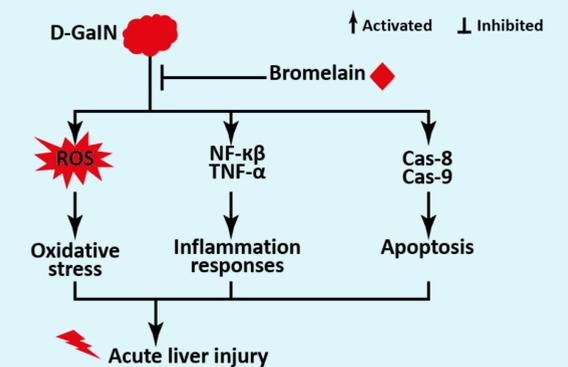
Each value represents the mean of 6 experiments ± S.E.M.

Statistical analysis was done using One-Way ANOVA followed by Tukey's post-hoc test.

*p < 0.05 vs. normal, @p < 0.05 vs. GalN, # p < 0.05 vs. SLM+ GalN

CONCLUSION:

These results demonstrate a significant hepatoprotective effect of bromelain against D-galactosamine-induced acute liver injury. Interestingly, Brom₄₀ was more effective than silymarin in ameliorating this type of liver injury.



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