In-vivo antiulcer activity This study aimed to evaluate the protective potential of ethyl acetate extract and isolated pure compounds against stomach ulcers caused by pylorus-ligation and ethanol-induced ulcers. The researchers induced gastric ulceration in animals by administering ethanol (1 mL/200 g b. w.) orally and by pylorus ligation using pentobarbitone (35 mg/kg i.p.). 2.7.1. Ethanol-induced ulcer Disease control (ethanol), positive control (ranitidine) and, test samples {ZA/Ea, ethylacetate extract (250 mg/kg); ZA/Eb, ethylacetate extract (500 mg/kg); ZA/1a, tambulin (25 mg/kg); ZA/1b, tambulin (50 mg/kg); ZA/2a, ombuin (25 mg/kg); ZA/2b, ombuin (50 mg/kg)} were given to the rats twice a day orally for 5 days prior to ulcer induction. According to the method described by Hollander et al., 1985, rats were administered with ethanol orally on 6th day (1 mL/200 g, 1 h) to induce uniform gastric ulcers and ranitidine was given as positive control at the dose of 50 mg/kg, orally. After the rats were euthanized, their stomachs were examined along the greater curve to assess the presence of ulcers. To determine the ulcer index, the length and width of ulcers in the glandular part of the stomach were multiplied (mm 2 /rat). This method provides a reliable and efficient way of analysing the effects of ethanol on the development of gastric ulcers in rats. Ulcer index protection was determined by the following formula: %Ulcerinhibition = (Ulcerindex Control ×100/Ulcerindex Ulcerindex Control 2.7.2. Pylorus ligated- ulcers Test ) Disease control (induced due to accumulation of hydrochloric acid), positive control (ranitidine) and test samples, {ZA/Ea, ethylacetate extract (250 mg/kg); ZA/Eb, ethylacetate extract (500 mg/kg); ZA/1a, 3 JournalofKingSaudUniversity-Science36(2024)103326 4 tambulin, (25 mg/kg); ZA/1b, tambulin (50 mg/kg); ZA/2a, ombuin (25 mg/kg); ZA/2b, ombuin (50 mg/kg)} were given to the rats for 5 days, and then they were kept in a cage for 18 h without food. Pento barbitone (35 mg/kg, i.p.) was used for sedation of animals. The belly was then opened and the pylorus was tied off without hurting the ani mals' blood supply. It was carefully brought back in place, and the wall of the belly was closed in two layers with stitches that were broken so often. After the surgery, the animals were not given any water (Shay et al., 1945). After 4 h, the animals were sacrificed, and the stomach was carefully taken out and cut along the larger curve. It was then carefully washed with 5.0 mL of 0.9 % NaCl, and ulcers were scored in the glandular part of the stomach by someone who wasn't part of the experiment. The ulcer index was estimated by adding the number of ulcers on each stomach and how bad each ulcer was. The method given by Sanyal et al. (1982) was used to figure out the ulcer score for the whole group. 2.8. Measurement of inflammatory cytokines 2.8.1. IL-1, TNF-α, and IL-6 Were measured using an ELISA kit within 2 hrs, and the concentra tion was calculated by following the standard procedure. Leukocyte infiltration was observed in the deep mucosal layer. A homogenate was prepared from the detached scarred fraction. The supernatant collected from the homogenate was used for further studies of interleukins (Almasaudi et al., 2016). 2.9. Histopathology Histopathology was examined by rats' stomach which were fixed in 10 % neutral formalin, then embedded in paraffin blocks. The eosin and hematoxylin stains were used for the section visibility. The degree of stomach damage was determined using the standard procedure mentioned in MICRON desquamative changes, mucosal defects of varying depths, and the degree of infiltrative changes. 2.10. Gastric wall mucus measurement Corne et al. (1974) developed a method for measuring gastric wall mucus in ethanol-induced ulcerated mice. Glandular segments from stomachs were isolated, weighed, and then incubated for 2 h in a 1 % Alcian blue

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solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) in test tubes. After centrifuging (100 g) the Alcian blue binding extract for 10 min, the absorbency of the supernatant was determined at 498 nm. The amount of Alcian blue taken from the glandular tissue was then determined (μg/g of glandular tissue). 2.11. Statistical analysis The obtained data were processed to determine the significant difference between the groups. Standard mean and error were optimized. To summarise the results, a one-way analysis of variance (ANOVA) was employed. Version 5.01 of GraphPad PRISM (GraphPad Software, Inc., USA) was used. The value p

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