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Evaluation of Gastroprotective Effect of Vanadyl Sulfate and Lycopene on rat model with Ethanol-Induced Gastric Mucosal Lesions

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Gastric ulcers result from an imbalance between endogenous defense mechanisms and certain aggressive agents. Many drugs were used to overcome this imbalance, but few literatures made on plants. Therefore, we try to evaluate the gastroprotective efficacy of two nutritional supplements (Vanadyl sulfate and Lycopene) in comparison to Lansoprazole. Five groups of seven healthy albino male rats each were received an oral daily dose of above agents for ten days. Then 1.25 ml of 95% ethanol orally used to induce mucosal injury and animals were sacrificed 1 hour later. Glutathione and malondialdehyde were estimated. A significant elevation in glutathione level found in Vanadyl and Lycopene-received groups in comparison to lansoprazole-received group (717.13±19.47 ¼mol/gm wet tissue, 609.55±17.6 ¼mol/gm wet tissue and 512.07±25.32 ¼mol/gm wet tissue respectively), with a significant reduction in malondialdehyde level (10.63±0.92 nmol/gm wet tissue, 12.66±0.56 nmol/g wet tissue and 14.90±0.33 nmol/gm wet tissue respectively). This revealed gastro-protective effects of Vanadyl and Lycopene in ameliorating the oxidative cellular damage.

Keywords: Vanadyl Sulfate, Lycopene, Gastric Mucosal Lesions.

Gastric mucosal injury generally results from an imbalance between the corrosive action of acid-pepsin and the mucosal integrity which is maintained by endogenous defense mechanisms such as glutathione (GSH), gastric mucus, bicarbonate secretion and prostaglandins[1]. This imbalance may also be caused by Helicobacter pylori[2], starvation[3] and ingestion of some ulcerogenic drugs like aspirin[4], indomethacin[5], and ethanol[6].These aggressive factors lead to[1] significant gastric mucosal damage[2] decreased tissue glutathione levels (the major cellular antioxidant that prevents cell from damage)[73] increased malondialdehyde levels (a product of the decomposition of polyunsaturated fatty acids during cellular oxidative stress) [84] increased myeloperoxidase enzyme (MPO) levels [9]. According to traditional medicine, Ibn Senna; the well-known and greatest philosopher used some food to relieve stomach aches. Nowadays, the ingredients of this food are known as nutritional supplements such as vanadyl sulfate and lycopene.

Therefore, it is of interest to evaluate the protective effects of these two nutritional supplements in comparison with lansoprazole in ethanol-induced gastric lesion model in albino rat. Vanadyl sulfate (the oxidative form of the trace element vanadium) is a popular muscle building product, produces a significant anabolic effect through promoting muscle uptake of glucose[10] and is found in eggs, carrots, soybeans and oats. Lycopene is a bright red, fat-soluble carotenoid pigment and is found in tomatoes, watermelon, papaya, and red guava. It exerts an anti-mutagenic effect via reducing the oxidative damage to DNA in humans[11].

METHODS

Materials

They were bought from Al-Madinah known drug bureau (lansoprazole was procured from Actavis /Barnstaple/UK, Vanadyl sulfate from Aldniuds Co./Germany, and lycopene from Liptis Pharmaceuticals /USA). The kits for biochemical estimations were procured from Fluka /Switzerland**,** Sigma /St.Louis / USA and from Biolabs SA/Maizy/France.

Acute toxicity studies

They were carried out according to (1) the LD_{50} of 95% ethanol was 6 ml/kg p.o.[12] (2) the safe American recommended dose of oral vanadyl sulfate is 5 mg/kg/day [13] and (3) lycopene has been given in doses as high as 2000mg/kg/day without any adverse effects[14]. However, we decided 56% ethanol as 8g/kg once daily[15] and 1/10th of upper doses of vanadyl sulfate and lycopene to be considered for the experiments[16].

Experimental design

The protocol of the experiment was approved by Al-Nahrain College of Medicine/ Animal Ethics Committee (Approval No. AEC/31/16/CMANU).

Animals

Thirty-five healthy male albino rats (200-220 grams) supplied by Al-Nahrain College of Medicine, were used after five days of standard housing conditions. The rats were distributed randomly into five groups (n=7) receiving a daily oral dose for ten days of : 1.5milliliter of distilled water for Group I as a negative control, 1.5milliliter of distilled water for Group II, lansoprazole 30mg/ kilogram body weight for Group III as a positive control, vanadyl sulfate 0.5mg/ kilogram body weight for Group IV and finally lycopene 200mg/ kilogram body weight for Group V .

On 11th day (at 3.30 p.m), the animals fasted for 18 hours. At 9.30 a.m of next day, a single dose of 56% ethanol as 1.25milliter p.o was given to all rats except Group I. The animals were sacrificed 1 hour later and their stomachs were separated. Then, by a longitudinal incision, gastric mucosa was bared and washed with normal saline to be prepared for examinations.

Histological study

After 48 hours of submerging in formalin, the specimens were desiccated, cleared and fixed in paraffin. The cut sections were stained with H&E dye to be examined under a polarized microscope.

Ulcer index

The gastric specimens were laid flat and the lesions (in the form of hemorrhage or linear breaks on the glandular portion of gastric mucosa) were measured using a dissecting microscope (Hamburg/Germany; 10xs) with a square grid[17]. Then the ulcer index of each specimen was calculated[18].

Biochemical study

Assay of mucosal glutathione [19]

After rinsing with cold saline, the specimen was sunken in alkaline solution (pH 8) for 5 minutes to maintain mucosal integrity. Then Acivicin was added to preserve glutathione activity. After centrifuging the sample, the supernatant was safeguarded at 4°C for 30 minutes during which 0.5 milliliter of trichloroacetic acid was added. By using spectrophotometer, glutathione level in each supernatant was measured.

Assay of malondialdehyde[20]

After rinsing with cold saline, the specimen was put in10 milliliter of potassium chloride (KCl) solution for 45 minutes to have a homogeneous solution. (0.5 milliliter) of this homogenate was added to a mixture of (Sodium dodecyl sulfate [0.2 milliliter]+ acetic acid [1.5 milliliter] + 2-thiobarbituric acid [1.5 milliliter] + distilled water [0.3 milliliter]). two minutes later, the whole mixture was incubated at 98°C for 1 hour. Then mixture was cooled in an ice-containing baker for 10 minutes. At this time, 5 milliliters of *n*-butanol: pyridine (15:1) was added. After centrifuging for 10 minutes, malondialdehyde level in each supernatant was measured using High

Performance Liquid Chromatography (HPLC) with fluorescent detection based on 2-thiobarbituric acid assay.

Statistical analysis

Data were analyzed using SPSS 13 software (IBM Corp., Armonk, N.Y., USA) as one-way ANOVA followed by student's t-test. The results were reported as mean ± SEM. And P<0.05 was considered statistically significant.

RESULTS

Induction of gastric lesions by ethanol 95% when was given orally in rats, was found to be approximately of 100% (group II). The obtained results from group IV (Vanadyl sulfate-received) and group V (lycopene-received) revealed significant elevation in GSH level (prevention index) as compared with group III (lansoprazole-received) and this equal to 75.38 ±2.66, 50.31± 4.78 and 91.06 ±0.25 respectively (Table I). All tested and control drugs showed a high significant reduction in free radicals of gastric tissue extract through increasing GSH and decreasing MDA levels (Table II).

DISCUSSION

Gastric mucosal lesion is formed when aggressive factors go beyond the cellular self-defense mechanisms. This leads to an excessive generation of reactive oxygen species (ROS) that promote degradation of the epithelial membrane components causing mucosal damage to the acinar part of the stomach[21] while the non-acinar portion remained relatively intact[22]. Some natural substances for example Emblica Officinalis have been known to strengthen gastric defense in healing induced gastric ulcers, enhancing cellular detoxification mechanisms and repairing the damaged non-proliferating cells[23]. Therefore, our study evaluated two dietary supplements (vanadyl sulfate and lycopene) compared to Lansoprazole (a proton pump inhibitor) that has a significant gastric mucosa protecting effect. The results showed that vanadium sulphate and lycopene exhibit a gastro-protective effect on ethanol-induced ulcers by increasing the Prostaglandins E2 production which in turn increasing the reduced GSH levels that responsible with sticking to ROS for wash out[22].

Table 1. Effect of lansoprazole, vanadyl sulfate and lycopene on ethanol -induced gastric ulcer parameters in rats

Groups	No. of ulcers	Total ulcer area (mm^2)	Ulcer index (100%)
I (negative control)	——	——	——
II (Ethanol only)	8.86±0.4	129.47±07.14	96.41±0.23
III (Lansoprazole + ethanol)	0.72±0.19**	23.10±10.21**	17.63±0.14**
IV (Vanadyl sulfate + ethanol)	2.29±0.36**	51.39±07.14**	39.22±0.16**
V (Lycopene + ethanol)	5.72±0.95**	87.57±12.40**	67.28±01.73**

Data are expressed as: mean ± Standard error of the mean, *group no.* =7. **P*<0.05, ***P*<0.01. mm^2 ; millimeter square

Table 2. Effect of lansoprazole, vanadyl sulfate and lycopene on gastric lesion healing parameters in rats

Groups	GSH level (µmol/g wet tissue)	MDA level (nmol /g wet tissue)
I	701.16±11.09	8.59±0.26
II	469.01±10.63	19.82±0.72
III	512.07±25.32*	14.90±0.33**
IV	717.13±19.47**	10.63±0.92**
V	609.55±17.64**	12.66±0.56**

Data are expressed as: mean ± Standard error of the mean, *group no.* =7. **P*<0.05, ***P*<0.01. GSH ; glutathione. MDA ; malondialdehyde. ¼mol/g; micromole per gram nmol/g ; nanomole per gram.

CONCLUSION

Vanadium sulphate and lycopene, through their property of ROS suppression, appear to improve the destructive effects of ethanol on the gastric mucosa.

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