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The Anti-Ulcer and Anti-Hypothyrodism Effects of Proanthocyanidin and Ranitidin in Female Rabbits With Gastric Ulceration Induced by Indomethacin

KEYWORDS: Proanthocyanidin, Ranitidine, Indomethacin, Thyroid hormones, Gastric Ulcer, rabbits

Abrar S. Abdul -Razak	Muna H. AL-Saeed	Eman A. AL-Masoudi
Department of Physiology and Pharmacology, College of Pharmacy, University of Basrah, Basrah, Iraq.	Department of Physiology and Pharmacology and Chemistry, College of Vet. Med. University of Basrah, Basrah, Iraq.	Department of Physiology and Pharmacology and Chemistry, College of Vet. Med. University of Basrah, Basrah, Iraq.

ABSTRACT

The present study aimed to evaluate the anti-ulcer activity of proanthocyanidin extracts of the grape seeds (Vitis vinifera) by using models of acute gastric lesions in rabbits induced by indomethacin. In addition to, attempt had been done to study the effect of proanthocyanidin on thyroid gland dysfunction caused by indomethacin . Thirty mature female rabbits were divided into five groups of equal number(6) as the following:: Group1:- healthy (negative control group) was administered normal saline (0.9 of Nacl) for 10 days; Group 2: was given indomethacin 75mg\kg B.W. for two days(positive control group); Group 3:- was administered indomethacin 75mg\kg B.W. for two days, then treated with proanthocyanidin(PA) 100mg\kg B.W. for 10 days; Group 4, was given indomethacin 75mg\kg for two days, then treated with proanthocyanidin(PA) 200mg\kg for 10 days; Group 5, was given indomethacin 75mg\kg for two days, then treated with ranitidin 50mg\kg for 10 days. The results showed that proanthocyanidin(PA) and ranitidin caused significant reduction (P≤0.05) in gastric volume, ulcer area with significant increase (P≤0.05) in gastric pH, and inhibition 100% and 71.09% of gastric ulceration female rabbits treated with (proanthocyanidin in a dose of 100mg/kg & 200mg/kg) and ranitidine respectively compared with negative control group. In addition to, it also revealed significant increase (P≤0.05) of TSH in female rabbits treated with indomethacin compared with the negative control and another groups. Indomethacin caused significant decrease (P≤0.05) in T3 and T4 compared with negative control group and another groups. It is conclude that proanthocyanidin extract of the grape seeds (Vitis vinifera) has good antiulcer activity and high effectiveness in ameliorating the hypothyroidism-induced by indomethacin.

Introduction:

Gastric ulceration is a benign lesion in the mucosal epithelium due to exposure of the stomach to excess acid and aggressive pepsin activity(1). Previous studies have shown that pathophysiology of gastric ulcer was an imbalance between mucosal defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) and injurious factors (acid, pepsin, bile and ***H. pylori***) in stomach(2). In addition to many factors include stress, smoking, nutritional deficiencies, ingestion of NSAIDS(3).

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used medication for curing of inflammatory diseases because of their effectiveness in alleviating swelling, pain of inflammation, fever and headache (4), but they have high incidence of gastrointestinal ulceration(5) and bleeding. Indomethacin is non- steroidal anti- inflammatory drug (NSAIDs) with analgesic and antipyretic effect(6), cause gastric erosions and ulcerations by inhibition of the cyclooxygenase enzyme(COX-1 and COX-2) which cause decrease of endogenous prostaglandins(7), increased production of reactive oxygen species and inducing lipid peroxidation (8). Many synthetic drugs are available for treatment of gastric ulcer such as proton pump inhibitors (omeprazole and lansoprazole), histamine receptor blockers(ranitidine and famotidine), antacids and parasympathetic blocker (pirenzipine and telezpine)(9,10). In spite of effectiveness of these drugs but they have various side effect (11). In recent years, using of alternative therapies and natural products, especially those derived from plants attract attention of scientist (12,13). They show importance of plant materials by-products that are specially rich in polyphenols and have a wide range of biological activities. Proanthocyanidins are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers, and bark (14). They are a class of phenolic compounds which take the form of oligomers or polymers of polyhydroxyl flavan-3-ol units, such as (+) catechin and ()-epicatechin (15). Proanthocyanidins oligomers have many-health-promoting effects including the ability to increase intra-cellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals (16). Indeed, grape seeds proanthocyanidin is a potent antioxidant with 50 times more antioxidant power than vitamin E and 20 times more than vitamin C(14, 17, 18). Thyroid hormones are important hormone for normal mammalian development and play important roles in the cardiovascular, nervous, immune and reproductive systems (19,22).

Indomethacin is most common drug used as experimental inhibitors of PG synthesis, these drugs can inhibit TSH secretion by acting directly at the pituitary gland (23)

The aim of this study is to evaluate the effect of proanthocyanidin and ranitidine on thyroid gland activity in female rabbits with gastric ulceration .

Materials and Methods

Drugs and Chemicals:

Indomethacin obtained from Safa co. Diala-Iraq, and ranitidine provided from GlaxoSmithKline, S.A. Aranda de Duero, Spain were suspend in 2ml of normal saline.

Serum concentration of T_3, T_4 and TSH were determined

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by using commercial ELISA kits Monobind Inc. lake forest CA 92630, USA.

Plant Material
Proanthocyanidin had been extracted from black Grape seeds that were used in this study. The black grape was hand-picked from local market with full skin intact. It was washed with tap water, the skin and fleshes were removed and the seeds are dried .The seeds of the grape were turned to powder with the help of an electric grinder and kept in dark container at 25c°.

Preparation of proanthocyanidin extract from grape seeds
Fifty grams of dried grape seeds powder was defatted with (500 ml) of n-hexane for 2hours by soxhlete. The combined n-hexane extract was concentrated below 50°C under reduced pressure in a rotary evaporator to get 7ml of yellow oily mass. This mass was dried at room temperature and further (40 gm.) was refluxed in (500ml) methanol (80%) in water with 3% hydrochloric acid for one hour then filtered by Buchner funnel and filter paper (Wattman No.185) .The filtrate was extracted with an equal volume of chloroform to remove pigments. The alcoholic layer was extracted with an equal volume of ethyl acetate treated with 2% of hydrochloric acid ,the ethyl acetate layer was concentrated by rotary evaporator at 45°C and dried at room temperature (24-25). The resultant extract (2.5gm) was pink colour and dry material .The extract was kept in dark glass container at 4°C.

Figure (1): The steps of preparation of proanthocyanidin

Experimental Animals
Thirty adult female rabbits with weigh ranged between (1500-2000.0mg) kept for an adaptation period for 1 month in the animal house of Veterinary Medicine College / Basrah University. The experimental animals were kept in individual cages, provided with ration composed of fodder in addition to green alfalfa (Medicago ***sativa***) and tap water ***ad libitum*** and given a prophylaxis drug against coccidiosis (Amprollium 1g/L of drinking water).

Experimental design
The rabbits divided into five groups(6) animals in each group as the following:

Group1:- healthy (-ve control group) orally administration 3ml of normal saline (0.9 of NaCL) for 10 days.
Group 2:- orally administered with indomethacin 75mg\kg B.W. for dissolve with 3ml of normal saline for two days(+ve control) group and remain without treatment for10 days.
Group 3:- treated with indomethacin 75mg\kg B.W. dissolved with 3ml of normal saline for two days, then treated with proanthocyanidin 100mg\kg B.W. dissolved with 3ml of normal saline for 10 days.
Group 4:- treated with indomethacin 75mg\kg B.W. dissolved with 3ml of normal saline for two days, then treated with proanthocyanidin 200mg\kg B.W. dissolve with 3ml of normal saline for 10 days.
Group 5:- treated with indomethacin 75mg\kg B.W. dissolved with 3ml of normal saline for two days, then treated with ranitidine 50mg\kg B.W. dissolved with 3ml of normal saline for 10 days.

Induction of gastric ulcer:
Gastric ulcers were induced in twenty four non starved rabbits by giving indomethacin (Safa co. Diala-Iraq) orally by one ml size syringe and in a dose of 75mg\kg for two days.

Collection of Blood Samples:
Blood samples (15ml) were collected from each animals at end of experiment from heart (cardiac puncture). The blood was deposited into tube without anticoagulant and then the blood samples were centrifuged at (3000 rpm) for 15 minutes and serum samples stored in polyethylene eppendorff tubes at (-20°C),which is used for studying hormonal analysis (TSH,T_3 and T_4).

Study parameter:-
Gastric ulcer index:
The method described by (26) employed in the present study. The stomachs opened along the greater curvature, washed with saline and examined by magnifying class for gastric ulcers observation .The sum of length for all lesions area for each animal was measured and served as the ulcer index . The curative ratio was calculated for each group using following equation:

Curative ratio (CR) = (LC-LT/LC) x100.

LC: The length of gastric ulcer in positive group.
LT: The length of gastric ulcer in treated group.

Determination of Gastric Juice Volume:
Gastric juice collected from each animal was centrifuged at 3000 rpm for 10 minutes to remove any solid debris, the volume of the supernatant was measured by graduated cylinder.

Determination of Gastric Juice Acidity

Acidity degree (pH) of gastric juice was determined by using pH meter apparatus (HI 9021).

Histological Techniques
The animals were sacrificed from all groups at the end of the experiment (after12 days from the beginning of the experiment) stomach and thyroid gland were fixed in 10% buffered formalin, dehydrated progressively in an increased ethanol concentrations, treated with xylene and embedded in paraffin. Five microns thickness sections of paraffin-embedded tissue were mounted on glass slides and stained with Hematoxyline and Eosin stain (H & E stain)(27-28).

Statistical Analysis:
The results of the present study were analyzed by using two-way covariance (ANOVA) test in all study. All statistical calculations were carried out by the aid of the statistical package SPSS V. 11 (SPSS Inc.). The data were expressed as means ± standard deviation (X ± SD). Least significant different test (LSD) was calculated to test difference between means of groups and subgroups(Stat soft, 2006).

Results
The obtained results in Table (1) revealed significant decrease ($P \leq 0.05$) in gastric volume in female rabbits with gastric ulceration treated with (PA at a dose 100mg/kg), (PA at a dose 200mg/kg) and ranitidine at a dose 50mg/kg

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compared with(+ve) group. Also gastric volume of gastric ulceration female rabbit treated with (PA at dose 200) revealed a significant decrease (P≤0.05) compared with (-ve) control and another groups while gastric volume of gastric ulceration female rabbit treated with (PA at dose 100mg/kg) and ranitidine revealed non-significant compared with (-ve) control group.

The results of gastric pH in female rabbit with gastric ulceration treated with (PA at a dose 100mg/kg), (PA at a dose 200mg/kg) and ranitidine at a dose 50mg/kg showed a significant increase (P≤0.05) compared with (+ve) control group.

The results of ulcer area showed significant reduce (P≤0.05) in gastric ulceration in female rabbits treated with(PA at dose 100mg/kg), (PA at dose 200mg/kg) and ranitidine compared with (+ve) control group. The results of ulcer area showed inhibition 100% in gastric ulceration female rabbits treated with (PA at dose 100mg/kg), (PA at dose 200mg/kg) and showed non-significant change compared with control group while the ulcer area showed 71.09% in gastric ulceration female rabbits treated with Ranitidine and showed significant increase (P≤0.05) compared with (-ve) control group.

The results of serum TSH, T_3 and T_4 concentrations have been presented in the Table (2). The results indicated that the TSH concentration was significantly (P≤0.05) increased during studied period in serum of gastric ulceration female rabbits (+ve control) compared with (–ve control) and other treated groups. Serum concentration of T_3 was significant (P≤0.05) decreased in serum of gastric ulceration female rabbits (+ve control) compared with (–ve control) and other treated groups.

The same pattern was found in the T_4 concentration which was significant (P≤0.05) decreased in serum of female rabbits with gastric ulceration (+ve control) and female rabbits with gastric ulceration treated with ranitidine compared with (–ve control) and other treated groups (PA at a dose 100mg/kg and PA at a dose 200 mg/kg).

Table (1) Effect of Proanthocyanidine and Ranitidine on Gastric Volume, Gastric PH, Ulcer area and Inhibition Percentage in Female Rabbits with gastric ulceration induced by Indomethacin (Mean ± SD) (n=6)

Parameters / Treatment	Gastric Volume (ml)	pH of Gastric Content	Ulcer area	Inhibition%
Control (-ve) Normal Saline(0.9% NaCl)	12.6±2.30 BC	2.42±0.30 A	0.00±0.00 C	-
Control (+ve) Indomethacine(75mg/kg)	22.08±3.42 A	1.40±0.22 B	7.37 ± 2.35 A	-
Indometh+PA(100mg/kg)	13.91±1.90 B	2.67±0.28 A	0.00 ± 0.00 C	100
Indometh+PA(200mg/kg)	9.00±1.04 D	2.70±0.41 A	0.00±0.00 C	100
Indometh+Ranitidine (50mg/kg)	11.25±1.54 C	2.46±0.12 A	2.13±1..17 B	71.09

N=number of animals., Capital letters denote differences between groups, P≤0.05 vs. control
PA= proanthocyanidin

Table (2) Effect of Proanthocyanidine and Ranitidine on Serum Concentrations of TSH, T3 and T_4 in Female Rabbits with Gastric Ulceration by Indomethacin (Mean±SD) (n=6)

Parameters / Treatments	TSH (µlU/ml)	T3 (ng/ml)	T4 (µg/dl)
Control (-ve) Normal Saline(0.9% NaCl)	2.15±0.30 B	1.40±0.20 A	7.66±1.08 A
Control (+ve) Indomethacine(75mg/kg)	3.40±0.41 A	0.63±0.19 B	2.88±0.54 C
Indometh + PA (100mg/kg)	2.28±0.32 B	1.36±0.17 A	7.00±1.30 A
Indometh + PA (200mg/kg)	2.28±0.38 B	1.41±0.34 A	6.75±0.93 A
Indometh + Ranitidine (50mg/kg)	2.21±0.58 B	1.25±0.28 A	5.41±1.24 B

N=number of animals., Capital letters denote differences between groups,P≤0.05 vs. control PA= proanthocyanidin

Fig.1:-Stomach of female rabbits (-ve) (Normal control).Showed normal gastric mucosa.

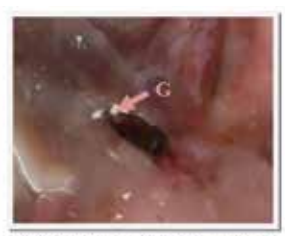

Fig.2:-Stomach of female rabbits (+ve control) treated with Indomethacin for 2 days induced gastric ulcer (GU). Showed gastric damage including gross mucosal lesion and haemarragic

Fig.3:-Stomach of female rabbits treated with (PA at a dose 100mg/kg) for 10 days. Showed no lesion at all tissue of stomach.

Fig.4:-Stomach of female rabbits treated with (PA at a dose 200mg/kg) for 10 days. Showed no lesion at all tissue of stomach.

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Fig.5:-Stomach of female rabbits treated with Ranitidine at a dose 50mg/kg) for 10 days. Showed redness at all tissue of stomach indicate presence of inflammation.

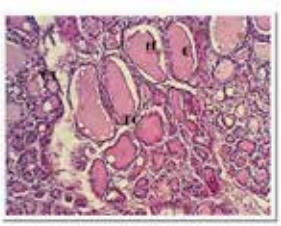

Fig.6 Section of thyroid gland of female rabbit (control negative). Showing normal architecture, thyroid follicles (tf), filled with colloid (C) lined by cuboidal thyrocytes (TC), parafollicular cells (CC), stain (H&E) 400X.

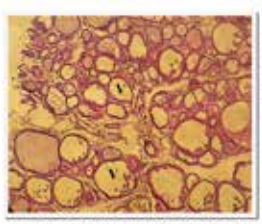

Fig.7 Section of thyroid gland of female rabbit with gastric ulceration , Showing several microfollicles, some with proliferating cell and hypertrophy of thyroid follicular, dilated and lined by flatted of thyrocyte (fl), vacuolation in some follicles (V) and depletion of parafollicular cells, stain (H&E) 400X.

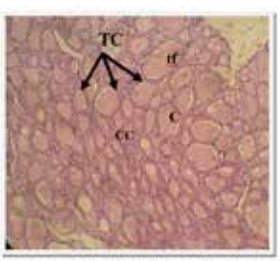

Fig.8 Section of thyroid gland of female rabbit with gastric ulceration treated with PA at dose 100mg/kg B.W. Showing normal architecture, thyroid follicles (tf), filled with colloid (C) lined by cuboidal thyrocytes (TC) (arrow), parafollicular cells (CC), stain (H&E) 400X.

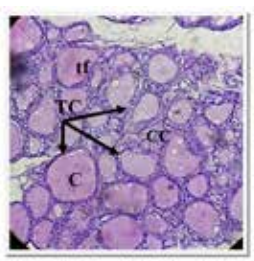

Fig.9 Section of thyroid gland of female rabbit with gastric ulceration treated with PA at dose 200mg/kg B.W. Showing normal architecture, thyroid follicles (tf), filled with colloid (C) lined by cuboidal thyrocytes (TC) (arrow), parafollicular cells (CC), stain (H&E) 400X.

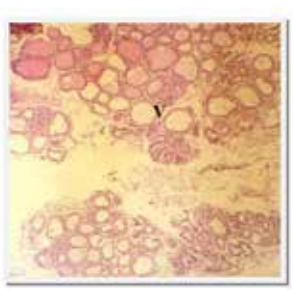

Fig.10 Section of thyroid gland of female rabbit with gastric ulceration treated with ranitidin at dose 50mg/kg B.W. Showing healing partially and some vacuolation (V) in follicles of thyroid gland, stain (H&E) 400X.

Discussion

Inhibitors of prostaglandin synthesis by gastric ulceration lead to decrease the level of TRH stimulation of serum TSH. The indomethacin lowered plasma prostaglandin E and F levels significantly. Gastric ulceration has effected on the serum TRH, TSH, T_3 and T_4 levels. These findings suggested that indomethacin blocks TRH responsiveness by a mechanism other than the inhibition of prostaglandin synthesis, probably by its previously demonstrated effect on increasing the fraction of unbound thyroid hormone.

One of the most common cause of gastric ulceration is consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) (29). Gastric ulceration induced by indomethacin is attributed mainly to different processes which include generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis and inhibition of prostaglandins(PGs) synthesis(30). Inhibition of PGs caused decrease in mucin secretion then allow hydrogen ions and pepsin to diffuse into the mucosa from lumen. So, back diffusion of acid and pepsin into the tissue stimulate more acid and pepsin secretion to cause more damage(31).Nowadays, the scientist interest in finding natural antioxidants from plant materials to replace synthetic ones for effective management of therapeutic drug toxicity such as peptic ulcer (32).

All animals administered with indomethacin showed reduced food intake, sluggishness, unthrifty appearance with some mortalities. Indomethacin caused gastric damage was further confirmed by the gross section in figure (2). Mortality occur may be due to gastric bleeding and perforation or may be due to hepatotoxicity of indomethacin (33).

Our present study provides a new understanding of the possible relationship between gastric ulcer and thyroid dysfunction. Indomethacin cause oxidative stress to thyroid gland, this seen clearly by histological section of thyroid gland as in figure(7) and measurement level of T_3,T_4 and TSH which commonly used as reliable indicators of the thyroid function in humans and experimental animals (34).

In present study, oral administration of indomethacin caused significant decreased in gastric pH, increase in gastric volume, total acidity and ulcer score. The ulceration induced by indomethacin is attributed mainly to different processes which include generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis and inhibition of prostaglandin synthesis(30). These findings are in agreement with (31). The significant increase in gastric volume following oral administration of indomethacin may be attributed to either free radicals formation or inhibition of prostaglandin synthesis which lead to increase gastric acid secretion(35) This result is agreed with(30, 36, 37) where they indicated that indomethacin have caused alterations in gastric secretions of rats. The significant decrease in gastric pH and significant increase in total acidity may attribute to decrease in mucin secretion allows hydrogen ions and pepsin to diffuse into the mucosa from lumen. So, back diffusion of acid and pepsin into the tissue stimulate more acid and pepsin secretion to cause more damage. Low pH value is a manifestation of increase hydrogen ion concentration in gastric juice (31). This finding is on line with (35).

Oral administration of proanthocyanidin (100mg\kg and 200mg\kg) significantly increased in gastric pH, and decreased in gastric volume, total acidity and ulcer score. Increasing gastric pH and decreasing total acidity proving anti-secretory activity of proanthocyanidin grape seed extract(38). In addition to, Grape Seed Extract has anti-histamine properties (it stabilizes the release of histamine from mast cells)(39-41). Proanthocyanidin decreased gastric volume because proanthocyanidin is antioxidant, cause lowering in the gastric secretion by acting on the gastric

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mucosa and inhibiting the generation of reactive oxygen species that initiate the oxidative stress in the gastric lumen (38).Number of ulcers score decreased due to decreased in gastric volume and total acidity, and increased in the pH (38). This findings are in agreement with (42)

Oral administration of ranitidin significantly increased in gastric pH , and decreased in gastric volume, total acidity and ulcer score. However, ranitidine decimate gastric acidity and increased in gastric pH due to its ability to block binding of histamine to H_2 receptor on parietal cell (43). Therefore ranitidin can counter the effect of indomethacin on gastric acid secretion. These result is cited by (44-45).

In present study, oral administration of indomethacin caused remarkably significant decreased in T3 and T4 and significant increase in TSH. Indomethacin had been caused thyroid dysfunction, this result was further confirmed by the histological section of thyroid gland in figure (7). This result on line with(46), they indicated that administration of indomethacin to rats causes an inhibition of thyroid function, measured by decreased thyroid hormone blood levels, without any change in the iodine organification process in these gland, where they administered indomethacin by an esophagic tube in two doses (total = 6 mg) given at 0 and 5 hours in experiment I and three doses (total = 9 mg) given at 0, 10 and 23 hours in experiment II. Significant increase in TSH after indomethacin administration is due to decrease level of T3 and T4 which stimulate TSH secretion where Pituitary TSH secretion is controlled by a negative feedback mechanism modulated by the circulating level of free T_4 and free T_3 and by conversion of T_4 to T_3 in the pituitary thyrotropic cells. TSH secretion is also influenced by thyrotropin releasing hormone (TRH), a 3-amino acid peptide synthesized in the hypothalamus which stimulates the pituitary to release TSH (34, 47, 48).

Oral administration of proanthocyanidin(100mg\kg and 200mg\kg) cause non-significant change in (T3,T4 and TSH) compared with (-ve) control group but significant increase compared with (+ve)group. This results due to improve function of thyroid gland. Euthyroid status after administeration of proanthocyanidin is due to antioxidant and scavenger of free radical (49).The protective effect of GSE treatment agreed with (50) who reported that oral intake of GSE reduced the oxidative stress. In addition, GSE treatment considerably increased the formation of antioxidant products which may be regarded to the phenolic constituents of GSE and its antioxidant activity. This result is supported by histological findings of thyroid glands in figure(8,9) which indicated markedly improvement functional status of thyroid gland after administration of proanthocyanidin at dose(100mg\kg and 200mg\kg) and measurement level of T_3,T_4 and TSH.

Oral administration of ranitidin cause non-significant change in (T_3 and TSH) compared with (-ve) control group but significant increase compared with (+ve)group. But it cause significant decrease compared with (-ve)control group. This indicate improve function of thyroid gland due to antioxidant (51) and potential oxygen radical scavenging function of ranitidin (52). The significant decrease in T_4 because peripheral deiodination of T_4 to T_3.This results are on line with (53) who indicated that no changes in TSH in 10 patients with peptic ulcer disease given oral ranitidine, Serum total and free thyroxin (TT_4 and FT_4) concentrations declined slightly, whereas total and free Triiodothyronine (TT_3 and FIT_3) increased slightly following ranitidine.

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