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Antioxidant and antiglycation effects of scopolamine in rat liver cells

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ABSTRACT

Tropane alkaloid, scopolamine is medicinally important compound produced by many plants of Solanaceous species. The present study was to investigate the antioxidant and antiglycation effects of this compound in culture of rat liver tissue. In this study, scopolamine at different concentrations were titred on rat liver cells. Then, the activity of glutathione peroxidase, superoxide dismutase and catalase as well as glyoxal and 2,2-diphenyl -1-picrylhydrazyl inhibition were measured by spectophotometry and changes in malondialdehyde (MDA) in liver cells were measured by high-performance liquid chromatography. The antioxidant and antiglycation activities of scopolamine increased as its concentrations increased in the liver cells, representing promotion of reactive oxygen species generation compared to control. Scopolamine exerts antioxidant and antiglycation activities in rat liver cells.

Key words: Free radical, Oxidative stress, Glycation, Scopolamine

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INTRODUCTION

Free radicals are active and highly unstable molecules which are derived from reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1-3]. Free radicals are produced by endogenous systems and exogenous sources in our body [4,5]. Increased production of free radicals leads to lipid peroxidation and oxidative stress, causing damages to the cells of various organs [6-8].

Liver is highly influenced by oxidative stress. ROS is constantly produced through metabolic processes and attacks to cell membrane lipids, proteins and DNA, leading to development of various diseases [9,10]. Antioxidant defense system is able to neutralize the outcomes of oxidative stress in the body, preventing damage to various organs [11-15]. Glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) are the most important components of antioxidant defense system against ROS. GPX, SOD and CAT that are highly active in liver scavenge superoxide and hydroxyl radicals [16,17].

Free radicals also contribute to glycation. The chemical reaction of amino groups of proteins with sugar-reducing carbonyl groups is called glycation or the maillard reaction. Through a series of complicated reactions, advanced glycation end products (AGEs) are finally produced [18]. AGEs formation is also enhanced by oxidative stress. Several studies have shown that AGEs are closely related to multiple diabetic complications [19,20]. Therefore, seeking for compounds with antioxidant and antiglycation properties will assist greatly in preventing oxidative-stress induced damages in biological systems and treating several diseases. Plants antioxidants have been shown to be effective in prevention and treatment of various oxidative stress induced diseases [21-29].

Scopolamine is medicinally important tropane alkaloid produced by many plants of Solanaceous species such as Anisodus, Atropa, Datura, Duboisia, Hyoscyamus and Scopolia [30]. This compound is used to treat several diseases thanks to analgesic, anti-inflammatory, antipyretic, anticonvulsant antidiarrhoeal and antioxidant activities. Plants containing this alkaloid have been used since ancient times as poisons, playing a vital role in ethnomedicine and orthodox medicine [31]. Recently, antioxidant properties of tropane alkaloids have been demonstrated, but their antiglycation activities have not been yet examined. The present study is aimed to investigate the antioxidant and antiglycation effects of scopolamine in culture of rat liver tissue.

MATERIALS AND METHODS

1. Chemicals

Standard enzymes [GPX (glutathione peroxidase), SOD (superoxide dismutase)and CAT(catalase), DPPH (2,2-diphenyl-1-picrylhydrazyl), scopolamine were purchased from Sigma Chemical Company (St. Louis, MO). Dulbecco's Modified Eagle Medium as culture media was obtained from Gibco (Paisley, Scotland). Other chemicals were of highest quality commercially available substances. The standard of scopolamine was dissolved in ethanol to make stock solutions. For each treatment, the stock solution was added to culture media solution to prepare the final desirable treatment concentration. Culture media and stock solution of scopolamine were freshly provided in distilled water and sterilized by filtration through a 0.22-µm filter (Acrodisc, Gelman). The amount of ethanol in the incubating solution was <0.1%. Subsequent experiments presented equivalent amount of ethanol; however, they had no clear effect on biological parameters.

2. Animals

In this study, pathogen-free male Sprague–Dawley rats (8-week-old), bred in the animal laboratory of Pasteur Institute, were used. The animals were maintained under normal conditions (12 hour light/dark cycle) and were fed with a standard laboratory diet and water.

3. Liver tissue culture

Rats were killed by decapitation and their liver was removed in sterile conditions. The liver lobes were washed twice with normal cold saline and then cut into small pieces. We added 0.5 gr of liver lobes in Nunclon dishes containing culture media, 30 mg/ml asparagine, antibiotics (200 U/ml penicillin and 200 mg/ml streptomycin) and 10% heat inactivated, fetal calf serum pH 7.4. Tissue cultures were incubated for 24 h at 37°C in a fully humidified atmosphere of 5% CO2 in air.

4. Experimental methods

This experimental study was done with two control and treatment groups. Normal liver tissue was used as control and scopolamine-treated liver tissue was used as treatment. The samples of liver tissue were placed in 45 separate petri dishes. The concentrations of 0, 0.5, 1, 2, 4, 8, 16 and 32 micromol of scopolamine were prepared separately.
The samples of liver tissue were treated with scopolamine at different concentrations in triplicate. Then, the treated samples were incubated for 24 h and prepared for later steps of the study.

4.1. Estimation of SOD activity

Measurement of SOD activity was performed spectrophotometrically as per the method of Sun et al [32]. By this method, xanthine-xanthine oxidase system is used to inhibit NBT reduction. One unit of SOD was determined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The activity of SOD was expressed as U/gr.

4.2. Estimation of GPX activity

GPX activity was measured by a modified method of Flohe and Gunzler [33]. A mixture containing 200 µL 1.5 mmol/l MgCl2, 200 µL 9.9 mmol/l isoascorbate, 200 µL 0.25 mM glutathione, 150 µL 1mM potassium phosphate buffer, 150 µL 1.5mmol/l NADPH, 150 µL 0.36 mM EDTA and 100 µl glutathione reductase (0.24 units) was added to 20 µl of tissue extract and incubated at 37°C for 10 min. GPX was measured at 340 nm for 1 min by the U2000 spectrophotometer. The enzyme activity was expressed as U/gr.

4.3. Estimation of CAT activity

The activity of CAT was measured according to Aebi's method [34]. The reaction mixture consisted of 200 µL 50 mM phosphate buffer (pH >5), 50 µL H2O2 and 10 µL tissue extract. The reaction was started by addition of the extract. The decrease in H2O2 was monitored at 240 nm and the enzyme activity was expressed as CAT units g-1 of tissue.

4.4. Malondialdehyde (MDA) assay

MDA, a product of lipid peroxidation, was determined by high-performance liquid chromatography [35]. and expressed as micromol MDA /mgr protein.

4.5. DPPH free radical-scavenging assay

DPPH is a stable free radical which causes changes in absorbance at 517 nm through absorbing electron from antioxidant compounds. The effect of the extract on DPPH radical was estimated [36]. Briefly, 100 µL of a series of extract concentration was mixed with 500 µL of 1 mM DPPH and then placed in the darkness for 30 min. The absorbance at 517 nm was measured and % inhibition calculated by spectrophotometer.

4.6. Glyoxal assay

Glyoxal content was measured using method described previously [37]. A mixture contained 100 µL sodium fumarate (pH=2.9). 100 µL girard was added to 100 µl of tissue extract and incubated at 37°C for 30 min. The absorbance was measured at 294 nm and glyoxal content was expressed in micromol.

5. Statistical analysis

Each experiment was run at least in duplicate and the data were presented as mean±SD. Statistical analysis of data was done by SAS version 9.2 using analysis of variance (ANOVA) using (Duncan's test). P value <0.05 was considered statistically significant for all experiments.

RESULTS

1. Antioxidant activity

Antioxidant enzyme activities at different (0.5 to 32 mmol/l) doses in liver tissue culture after 24 h are exhibited in Table1. Treatment of liver tissue with scopolamine significantly increased these enzyme activities at concentration of 32 mmol/l compared to control. As the concentration of scopolamine increased, CAT, GPX and SOD activities increased.

The ability to inhibit free radicals was investigated by DPPH (Table 2). In this experiment, as the concentration of scopolamine increased, free radicals were inhibited more pronouncedly as compared to the control. The highest inhibitory effect was observed with scopolamine at 32 mmol.

The oxidative damage products of lipids in liver tissue after exposure to 0.5 to 32 mmol/l scopolamine measured as MDA are exhibited in Table 2. Compared to baseline level of MDA in the control, 24-h treatment with scopolamine caused MDA to decrease in a dose-dependent manner.

2. Antiglycation activity

Scopolamine caused glyoxal base line level to decrease in a dose-dependent manner as compared to the control (Table 2).

Table 1. Activities of antioxidant enzymes in liver tissue after 24-h scopolamine treatment [a]

Scopolamine			
Concentration	SOD (units g^{-1})	GPX (units g^{-1})	CAT (units g^{-1})
0	3.8±0.37	4.6± 0.46	6.6±0.88
0.5	4.1±0.24	4.23±0.2	6.93±0.75
1	6.2±0.58	4.86± 0.5	7.06±0.19
2	8± 0.86	4.4±0.32	7.3±0.72
4	9.6±0.74	4.96± 0.4	8±0.5
8	11.8±0.76	5.7±0.45	9.4±0.73
16	14±0.86	6.8±0.5	10.3±0.78
32	#15.5±0.94	#7.7±0.45	#11.8±0.67

[a] Data are presented as mean±SD. (number of replicates=3).
[#] Significant increased in enzyme activity.

Table 2. The level of lipid peroxidation, inhibition of DPPH* and the amount of glyoxal in liver tissue after 24-h scopolamine treatment [a]

Scopolamine			
Concentration	MDA (nmol/mg))	DPPH (nmol/mg)	glyoxal (nmol/mg)
0	22.13±1.51	11.4± 0.94	31.5±0.96
0.5	18.5±0.96	12.1±0.86	30±0.65
1	14.2±0.96	13.3± 0.74	22.8±1.31
2	10.5± 0.75	18.1±0.77	16.6±0.94
4	9±0.74	21.3± 0.86	12.2±0.86
8	7.2±0.53	26.4±1.06	11±0.82
16	4.6±0.29	29.7±1.52	8.1±0.51
32	∞3.2±0.32	#36.1±1.4	* 7.4±0.57

2,2-diphenyl -1-picrylhydrazyl

[a] Data were presented as mean±SD. (number of replicates=3).

∞Significant alteration in amount of MDA.

 # Significant alteration in absorbance at 517 nm.

**Significant alteration in the amount of glyoxal.*

DISCUSSION

In this study, we utilized tissue culture media to investigate the effect of scopolamine on normal rat liver culture. Liver involves in various main functions containing synthesis of many compounds like albumin, globulin, clotting factors, catabolism of hormones, and detoxification of many compounds and accumulates of the materials absorbed from digestive tract [38]. In liver, reactive free radicals, implicated in many diseases, lead to damage to cell membranes, DNA and protein breakdown [39,40]. Human cells have two lines of defense against free radicals-endogenous antioxidants and scavenger enzymes. CAT, SOD, and GPX enzymes, abundantly found in liver tissue, are considered as the first defense lines of cells against free radicals [41]. SOD scavenges superoxide anion through transforming it into hydrogen peroxide and hence decreases its toxic effects. CAT is another antioxidant enzyme which is widely released in animal tissues and exerts the highest activity in the liver and red blood cells. CAT breaks down hydrogen peroxide and protects body tissues against hydroxyl radicals. GPX also mitigates H2O2 toxic effects in red blood cells [42]. Our findings indicated not only there was a baseline level of CAT, SOD, and GPX in liver tissue but also liver tissue treatment with scopolamine caused a significant increase in this enzyme' antioxidant activity.

Apart from these enzymes, DPPH experiments have been widely used to measure the antioxidant activities of plant extracts based on the ability to scavenge free radicals [43]. Our findings showed that scopolamine had potent radical scavenging activity. Our results are in line with the literature reporting that hexane extract indicated high antioxidant activities. Increase in the alkaloids such as hyoscyamine, scopolamine, and anisodine in the root of Anisodus acutangulus (from Solanaceous family) caused antioxidant effects of the plant to increase compared to the control [44] which confirms our study.

However, enhanced enzyme activities in the present study are consistent with other reports that have shown these enzymes were triggered by ROS. In the liver, significant alteration of CAT, GPX, SOD and GSH-Px activities have been shown after treatment with xenochemicals [28]. A family member of tropane alkaloids such as atropine and cocaine are commonly found in plants of different families. Aluminum caused increase in biosynthesis of secondary metabolites, such as scopolamine, and induction of ROS production and therefore increased enzyme activities of CAT and SOD. In this study the effect of aluminum at various concentrations on the level of secondary metabolites synthesis and enzyme activities was measured. It indicated that aluminum at 25 and 225 mmol caused tropane alkaloid in stem to reach maximum level and SOD and CAT exhibited the highest activity at 25 and 225 mmol, respectively [45].

Another example of oxygen free radical damage is lipid peroxidation, which can lead to liver dysfunction [6]. To better characterize whether lipid peroxidation was damaged by ROS, we measured the level of MDA. The formation of MDA is one of the oldest and most frequently used tests for measurement of lipid peroxidation by ROS. Detectable level of MDA was present in our cells, suggesting that a baseline level of lipids oxidation damage exist in normal liver tissue culture. Particularly, evidence shows that liver tissue damage after exposure to ROS inducer is accompanied by lipid peroxidation and increases throughout MDA formation. Our findings revealed that scopolamine decreased amount of MDA. Consistent with our study, berberine also promoted enzyme activity of SOD and mitigated superoxide ion and MDA amount [46].

Moreover, in this study we examined antiglycation activity of scopolamine. Glyoxal is a reactive α-oxoaldehyde that is a physiological metabolite formed by ascorbate autoxidation, oxidative degradation of glucose and degradation of

glycated proteins. As with methylglyoxal, glyoxal is capable of inducing cellular damage, but may also increase the rate of glycation, leading to the formation of AGEs. Our results showed that scopolamine had potent antiglycation activity and caused the amount of glyoxal in liver tissue to decrease. The present study is the first investigation of antiglycation effect of scopolamine. Our findings indicated that scopolamine had considerable antiglycation effect and caused glyoxal to decrease in liver tissue. As mentioned previously, ROS contributes to AGE formation and hence diabetes development [47]. Therefore, tropane alkaloids could be used in diabetes treatment. This recommendation needs further investigation. There are other plants with antioxidant activity which worth examining, too.

CONCLUSION

The present study indicates that scopolamine is bioactive compound with antioxidant and antiglycation activities. This could be supported by further research.

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