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Effects of Propolis on Serum Biochemical Parameters in Azaserine Treated Rats

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Abstract

Azaserine (o-diazoacetyl-L-serine) is an antimetabolite obtained from streptomycetes cultures and is used experimentally to produce atypical acinar cell focus (AACF) in rat exocrine pancreas. Propolis is a resinous hive product with antioxidant, anticarcinogenic, antimicrobial and anti-inflammatory effects that bees collect from the plants around them. In this study, we aimed performed to investigate the effect of ethanol extract of propolis (EEP) on serum biochemical changes in azaserin induced pancreatic cancer in Wistar rats. For this purpose, male Wistar albino rats were randomly divided into 4 groups, 10 in each group. AzCt and AzProp group 2-week-old male rats were given azaserine (30 mg/kg bw) intraperitoneally (ip). Propolis (EEP) (oral 80 mg/lt) was added to the drinking water of Prop and AzProp group rats. For the first time in this study, the effects of propolis on rats with neoplastic structures formed by azaserine were investigated in terms of biochemical parameters (AST, ALP, ALT, BILD, BILT, CHOL, LDH, TRIG, UREA, TAS, TOS and OSI). According to this; AzProp group values were compared to AzCt group, respectively, AST, UREA, TOS and OSI, there is a statistically significant difference in terms of values. As a result, it was seen that the oxidative stress caused by azaserine-induced neoplastic structures decreased with the use of propolis and that propolis had a positive effect on the measured biochemical parameters.

Keywords:
Azaserine, propolis, serum, oxidative stress

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Introduction

Cancer is one of the most important causes of death worldwide. It is one of the leading causes of death along with heart diseases. On a global scale, approximately 12 million people are diagnosed with cancer every year, 7 million patients die from cancer, and approximately 25 million people

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worldwide are living with cancer diagnosis today. It is estimated that there will be 1,688,780 new cancer cases in the United States in 2017 and 600,920 people will die of cancer (Siegel et al., 2017).

In this study, azaserine (O-diazoacetyl-L-serine), which was isolated from Streptomyces species fungal cultures for the first time, was used as a good carcinogenesis model. Azeserine is a toxic, mutagenic and carcinogenic compound. It is possible to inject rats with azaserine in accordance with the protocol of Longnecker & Curphey (1975), then to monitor neoplastic changes and developments in the pancreas, and to conduct studies on AACF in the early stages. The carcinogenic effect of azaserine is generally assumed to result from the inhibition of enzymes involved in DNA synthesis. Azaserine was an antibiotic previously observed in Streptomyces extracts, a known inhibitor of purine ribonucleotide biosynthesis. The azaserine-rat model is a useful model that allows to study neoplastic developments from early weeks (Baggott et al., 2007).

Propolis is a natural substance, also known as bee glue, produced by honey bees from sap, resin and mucilage collected from various parts of the plant and then mixed with various enzymes. Honey bees use propolis to repair the damage in the hive, to heal the inner walls, to keep the humidity and temperature constant in the hive, to protect the colony from pathogenic microorganisms and parasites (Iqbal et al., 2019; Zabaiou et al.; 2019; Stojanovi et al., 2020; Catchpole et al., 2015). Propolis is used by people in different fields, especially in folk medicine (Popova et al., 2017). It is known to have biological properties such as antibacterial, anticarcinogenic, antioxidant and anti-inflammatory (Catchpole et al., 2015; Przybyłek et al., 2019). It has been shown that propolis extract can affect cell proliferation, avoidance of apoptosis, angiogenesis and metastasis, which are key processes for cancer development. It has been observed that propolis protects healthy cells from the harmful effects of chemotherapy and radiotherapy in cancer patients. It has been stated that the use of propolis at certain intervals, rather than curing cancer, will be effective in preventing cancer (Sforcina & Bankovab, 2011).

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) parameters change especially in case of liver damage. Similarly, alkaline phosphatase (ALP), lactic dehydrogenase (LDH) are the parameters by which changes in cells can be observed. Several types of liver disease can cause a rise in gamma glutamyl transferase (GGT). Serum total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) are oxidative stress markers (Satue et al., 2022). Positive effects of propolis on these parameters were investigated in the presence of agents that cause neoplastic changes in cells, such as azeserin. In this study, it was aimed to investigate the effect of propolis on blood serum parameters in rats with experimentally generated AACFs.

Materials and Method

Experimental animals were provided by Hatay Mustafa Kemal University Experimental Research Application and Research Center (HMKÜ-DAM), and study approval was obtained with the ethics committee document dated 31/01/2019 and numbered 2019/01-4. Forty Wistar albino two-week-old male rats were used in the study. The rats were randomly divided into 4 groups of 10. Until the

end of 32 weeks, they were fed ad libitum with standard commercial rat pellet food in special rat cages, at an average room temperature of 22°C and in a light-illuminated environment.

Cnt: Control group fed only standard diet (n=10).

AzCt: Azaserine injected (30 mg/kg bw) and standard diet fed group (n=10) (Longnecker & Curphey, 1975).

AzProp: The group injected with azaserine (30 mg/kg body weight) from the 12th week and added ethanolic extracts of propolis (EEP) (80 mg/lt) to the drinking water. (n=10).

Prop: The group fed with a standard diet and to which ethanolic extracts of propolis (EEP) (80 mg of propolis, completing the volume to 100 ml with 70% ethanol), will be added to their drinking water. (Mani et al., 2006) (n=10).

Biochemical Analysis

Detection of ALT, AST, LDH, TAS, TOS, GGT, Cholesterol CHOL, direct bilirubin (BILD), Total bilirubin (BILT), Triglycerides (TRIG), and UREA from blood serum was performed with an autoanalyzer Siemens/Atelica and using Siemens commercial kits.

Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) Measurements

Total antioxidant capacity was measured using the Rel Assay kit developed by Erel (Rel Assay Kit Diagnostics, Turkey). Trolox, a water-soluble analog of vitamin E, was used as the calibrator. The results were expressed as mmol Torolox equiv./lt (Erel, 2004).

Total oxidant capacity was measured using the Rel Assay kit developed by Erel (Rel Assay Kit Diagnostics, Turkey). Hydrogen peroxide was used as calibrator. The results were expressed as μmol H_2O_2 equiv./lt (Erel, 2005).

Statistical Evaluation

Student- Newman- Keuls Multiple Comparison statistical analysis (ANOVA) method was used in the study (ProStat version 5.04 for Windows). The tests were conducted based on the statistical information method. It is given by taking the arithmetic mean and ± standard deviation of the test results, and $p< 0.05$ values were considered statistically significant.

Results

The biochemical analysis levels of the groups are given in Table 1. AST, TOS and OSI values of the AzCt group were found to be statistically significantly increased compared to the Cnt group ($p<0.05$). ALP and UREA values were significantly lower in the AzProp group than in the Cnt

group. Similarly, ALP, LDH and UREA values were significantly decreased in the Prop group compared to the Cnt group, while the CHOL value increased significantly between the same groups ($p<0.05$). When AzCt and AzProp group values were compared, it was observed that AzProp group values were statistically significantly decreased in AST, UREA, TOS and OSI values. Considering the AST, LDH, UREA and TOS values in Prop group, a significant reduction was observed compared to the AzCt group ($p<0.05$).

Table 1. Some biochemical parameters in blood serum of Control, Azaserine, Azaserine+Propolis and Propolis groups ($p<0.05$).

	Groups			
	Cnt	**AzCt**	**AzProp**	**Prop**
AST (U/L)	173.4 ± 15.7	232.4 ± 27.7*	175 ± 31.7**	176.6 ± 31.6**
ALP (U/L)	150.2 ± 36.3	126.3 ± 28.5	101.9 ± 15.8*	108.1 ± 29.3*
ALT (U/L)	65.60 ± 7.3	71.4 ± 2.3	68.2 ± 3.8	65 ± 4.6
BILD (mg/dl)	0.014 ± 0.007	0.018 ± 0.01	0.011 ± 0.003	0.013 ± 0.007
BILT (mg/dl)	0.025 ± 0.01	0.032 ± 0.013	0.031 ± 0.01	0.035 ± 0.01
CHOL (mg/dl)	34.66 ± 6.5	42.22 ± 6.8	38.20 ± 6.7	46 ± 7.05*
LDH (U/L)	1817.1 ± 612	1793 ± 303	1433 ± 433	1104 ± 200*/**
TRIG (mg/dl)	58 ± 17.4	51.5 ± 15.6	63 ± 21.5	67.5 ± 11.8
ÜREA (mg/L)	22.1 ± 1.1	21.6 ± 1.7	19 ±2.2*/**	19.37 ±0.9*/**
TAS (mmol/L)	1.41 ± 0.37	1.08 ± 0.15	1.20 ± 0.08	1.13 ± 0.06
TOS (μmol/L)	18.43 ± 2	25.62 ± 4.6*	19.58 ± 0.5**	17.10 ± 4.1**
OSI (AU)	0.155 ± 0.04	0.234 ± 0.06*	0.170 ± 0.005**	0.152 ± 0.03

* The group that differs from the Cnt group.
** The group that differs from the AzCt group.

ALT was highest in AzCt and lowest in Prop group. BILD was found highest in AzCt group and lowest in AzProp group. The BILT level was highest in the Prop group and the lowest in the Cnt group, and these changes were not statistically significant.

TAS values were highest in the Cnt group and lowest in the AzCt group. TOS values were highest in the Prop group and the lowest in AzCt but were not statistically significant. OSI value was highest in AzCt group. As a result of the administration of propolis, it was observed that the increase in OSI value caused by azaserine decreased and approached the control group.

Discussion

Environmental factors are important in the development of cancer. Among the most important of these factors are dietary habits and their interactions. The increasing cancer burden is linked to

population growth and aging, as well as social and economic development, including diet, nutrition and physical activity (Boyle & Levin, 2008).

Transaminase enzymes are considered, specifically AST and ALT. They can be biomarkers of hazardous liver activities and provide a quantitative measure of the extent of hepatocellular damage to a certain level. Propetamphos, an aliphatic organophosphate (OP) insecticide that inhibits acetylcholinesterase (AChE) enzyme activity in insect control in crops, decreased the elevation in glucose and triglyceride levels and AST, ALT and ALP activities induced by propetamphos alone. Similarly, in our study, the presence of propolis showed a reducing effect in the increased AST, ALT and ALP values due to azaserine. In this way, it is possible to say that propolis minimizes the toxic effects of azaserine (Çetin et al., 2010).

According to the results of a study conducted to investigate the serum biochemical effect of *Phyllanthus amarus* extracts on azaserine-induced pancreatic cancer, a significant change occurred in pancreatic amylase, AST, ALT and ALP activities after oral administration of *Phyllanthus amarus* extracts (Prajapati et al., 2015). In this study, it was reported that there was an increase in AST, ALT and ALP values in the AzCt group compared to the Cnt group. Similarly, in our study, it was observed that AST and ALT values increased in the AzCt group compared to the control group. High ALP value can be seen in cases related to liver damage. Similarly, a decrease in ALP values was observed in the groups given propolis compared to the AzCt group.

Azaserine is a well-known carcinogen reported to produce free radicals and widely used experimentally in pancreatic carcinogenesis in rats (Revathi et al., 2012). In contrast, propolis has an antioxidant activity that may be responsible for its free radical scavenging ability. (Catchpole et al., 2015; Przybyłek et al., 2019). In our study, as a result of this antioxidant effect of propolis, it was observed that the TAS value increased in the AzProp group compared to the AzCt group. Similarly, azaserine, known as a free radical source, increased the TOS value and caused the oxidative stress balance to shift in favor of oxidants (Prajapati et al., 2015).

In a study conducted to determine the harmful properties of methanol extract of Nigerian bee propolis , it was stated that administration of propolis did not cause a significant change in serum potassium ions, alkaline phosphatase and alanine aminotransferase activities (Shittu et al., 2015). However, it was stated that the level of total protein, urea, creatinine and sodium ions decreased significantly. Similarly, the decrease in urea level showed a significant decrease in both Cnt and AzCt groups in our study.

Our study has shown that ethanolic extracts of propolis can work as an antioxidant agent and thus have positive effects on cell serum parameters. Our study revealed that ethanolic extracts of propolis can be given as a supplement as an antioxidant agent. More studies are needed with propolis to investigate the benefits of correct dosage and application methods that are free of or minimized side effects.

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Author Contributions

H.Y. and Ü.M. performed all the experiments and drafted the main manuscript text. H.Y. and Ü.M. designed the experimental work, final versions of statistics table. H.Y. reviewed and approved the final version of the manuscript.

Conflict of Interest

The author declares that no conflict of interest.

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