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EVALUATION OF RABBIT KIDNEY AFTER ADDITION OF AMYGDALIN

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ABSTRACT

Amygdalin is non-toxic, but when placed with certain amino acids in the small intestine, it leads to the production of toxic hydrocyanic acid.
In our study, we observed the effect of amygdalin on the activity of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in the kidney after a short administration. The experiment was performed on male California meat rabbits of the P91 rabbit line (n = 20). Amygdalin was applied intramuscularly in the experimental group E1 and E2 daily for 14 days. Animals from experimental groups E3 and E4 were fed crushed apricot seeds. Apricot seeds were mixed into the feed mixture. The control group of animals was fed with a standard commercial feed mixture.
Significant differences ($p < 0.05$) were observed in GPx activity in the control group and experimental considerations E1 and E2, where amygdalin was administered *i.m.* A significant difference was also observed among the control group and the E4 group, where amygdalin was administered to the animals *per os*.
We also observed differences among SOD activity. Significant differences ($p < 0.05$) were found out in the control group and the experimental group E2 where amygdalin was administered *i.m.* and between the control group and the E4 group, where amygdalin was administered to the animals *per os*. We found significant differences between experimental groups E1:E2 and between experimental group E1 and experimental group E4. We did not find significant differences among the other groups ($p > 0.05$).
Many aspects of amygdalin administration have not yet been explored, requiring other research.

Keywords: amygdalin, Glutathione Peroxidase, Superoxide Dismutase, rabbit, kidney

INTRODUCTION

Amygdalin (D-mandelonitrile-b-D-gentiobioside) is one of the major pharmacological components present in fruits and seeds of Rosaceae plants such as apples, apricots, almonds, cherries, plums, and peaches (**Barceloux, 2009; Bolarinwa *et al.*, 2014**), alfalfa, bayberry (**Jaswal *et al.*, 2018; Guo *et al.*, 2013**). The *Rosaceae* is family characterized by cyanogenic glycosides. The family *Rosaceae* contains typical secondary plant products. According to **London-Shafir *et al.* (2003)** these secondary plant products have protective functions against herbivory. These compounds can produce toxic substances - hydrocyanic acid (HCN) affected by acid or appropriate hydrolytic enzymes. Most plants containing cyanogenic glycosides, also possess the enzymes necessary for their hydrolysis. After injured of the plant tissues substrates and enzymes are usually compartmented come together. This is that reason, the products of hydrolysis are thought to be the actual toxicants for herbivores.
Amygdalin is one of the most common cyanogenic glycosides.
Cyanogenic glucosides are widespread amino acid-derived bioactive natural products found out in more than 2,650 species (**Gleadow and Mølle, 2014; Thodberg *et al.*, 2018**) which consist of an aglycone and a sugar moiety (**Vetter, 2000; Bolarinwa *et al.*, 2014**).
Acording to **Bolarinwa *et al.* (2014)** cyanogenic glycosides, phytotoxins are natural plant toxicants. Cyanogenic glycosides produce HCN. Cyanogenic acid is a powerful and a rapidly acting poison. Cyanogenic glycosides including amygdalin, linamarin, dhurrin, taxiphyllin, and prunasin have been detected inedible plants. Amygdalin is also called bitter almond, apricot, laetrile. Amygdalin belongs to the aromatic cyanogenic glycoside group. Its molecular formula is: $C_{20}H_{27}NO_{11}$. The chemical structure is D-mandelonitrile-β-D-glucoside-6-β-glucoside (**Kolesár *et al.*, 2015**) and molecular weight is 457.43 (**Shi *et al.*, 2019; He, 2020**).
When is amygdalin alone, is nontoxic. Amygdalin digested by certain commonly enzymes found in the small intestine of humans such as β-D-glucosidase, produces toxic hydrocyanic acid. β-D-glucosidase is in various commonly consumed foods. Amygdalin and hydrocyanic acid potentially affect the digestive system and have been exert anti-asthmatic and antitussive effects, stimulate the respiratory centre (**Albogami *et al.*, 2020**).
Pharmacological activities of amygdalin have been well documented over the years, including anti-fibrosis, anti-inflammation, analgesia, auxiliary anticancer, immunoregulation, anti-atherosclerosis, anti-cardiac hypertrophy, anti-ulcer, and hypoglycaemia effects. It can also inhibit sperm hyaluronidase activity and mobility, induce ovulation, relieve endometriosis, and present the potential to treat neurodegenerative diseases (**He, 2020**).
In the 1970s it was one of the most popular, non-conventional, anti-cancer treatments and by 1978, 70,000 US cancer patients had used amygdalin (**Moss, 2005; Kolesár *et al.*, 2015**). Still, evidence-based research on amygdalin was and is sparse and its benefit controversial (**Kolesár *et al.*, 2015**).
The benefit of amygdalin is controversial. Many authors amygdalin regard as a natural cancer cure, but many authors are opponents warn that amygdalin is ineffective and even toxic **Zbyňovská *et al.* (2017)** claims that it has not been recorded in connection with amygdalin no serious acute toxicity. Many aspects of amygdalin administration have not yet been explored, making further investigation necessary (**Blaheta *et al.*, 2016; Kováčiková *et al.*, 2019**).
The results suggest that regular intake of bitter apricot seeds may be considered potentially useful for prevention of cardiovascular diseases (**Kopčeková *et al.*, 2018**).
The different ways of the cyanide metabolism are known in a living organism. Combining cyanide with hydroxocobalamin (vitamin B_{12}), to obtain cyanocobalamin (i.e., vitamin B12). The remaining cyanide ions are oxidized to formats and carbon dioxide. Individual formats are excreted in urine and through the lungs are excreted carbon dioxide and hydrocyanic acid (**Jaszczak-Wilke *et al.*, 2021**).
SOD converts superoxide (O_2^-) to hydrogen peroxide (H_2O_2) and three isoforms have been identified. GPx is responsible for the conversion of H_2O_2 and other organic peroxides, to water and oxygen (**Crawford *et al.*, 2011**).
Manganese-containing Superoxide Dismutase (MnSOD) is an essential primary antioxidant enzyme that converts superoxide radical to hydrogen peroxide and molecular oxygen within the mitochondrial matrix. Cytosolic Glutathione Peroxidase (GPx) converts hydrogen peroxide into water (**Li *et al.*, 2000**).

Measuring the activities of some antioxidant enzymes, such as superoxide dismutase (SOD), catalase or glutathione peroxidase, can be used to indirectly evaluate free radicals (**Ozdemir *et al.*, 2012**).
Because of physiological stress, pathogens, or inflammatory responses the heavily proactive oxidative metabolism may be formed. Many physiological factors and genetic diversity of individual affect the oxidative defence capacity of the individual. Such characteristics may bring about a predisposition in the pathogenesis of the disease in the persistence and the recurrence of symptoms (**Felicity *et al.*, 2008; Ozdemir, 2012**).
Aim of this study was investigated activity of Superoxide Dismutase and Glutathione Peroxidase in rabbit kidney after different application of amygdalin.

MATERIAL AND METHODS

Material

Male California meat rabbit line P91 (n = 20) were used for the experiment. The rabbits were kept on the Animal Production Research Centre Nitra of National Agricultural and Food Centre (Slovakia). The rabbits were kept in wire cages under welfare conditions. The ambient temperature was 20-24 °C, daylight 12 hours and average humidity 55 ± 10%. The animals were fed a commercial compound feed in the pellets form *ad libitum.* Access to water was also *ad libitum.* There were no deaths during fattening.
Amygdalin for experimental purposes was purchased from Sigma Aldrich (St. Louis, MO, USA). Amygdalin was injected into animals intramuscularly into the biceps femoris muscle. An appropriate amount of amygdalin was dissolved in saline prior to administration.
The apricot seeds used in the experiment was purchased from Trasco (Žiar n. Hronom, Slovakia). Seeds contained 5.2% amygdalin. The amygdalin content of the seeds was determined by TLC chromatography of a CS-9000 UV densimeter. The chemical composition of apricot seeds was performed according to **Gálik *et al.* (2014**).

Animal groups

For experiment animals were randomly divided to five groups. The first group was control group what was fed standard commerce feed mixture. Solution of amygdalin was applied by intramuscular injection to musculus of animals in experimental group E1 and E2 in concentration 0,6 and 3,0 mg/kg b. w. daily for 14 days. Animals from experimental group E3 and E4 were fed by crushed apricot seeds, at dose 60 and 300 mg/kg b. w. The apricot seeds were mixed into the feed mixture.
Institutional and national guidelines were followed for the care and use of animals, and all experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3 and Ethic Committee.

Methods

Tissue preparation was done according to **Jung and Henke (1996)** and **Jung *et al.* (1993)**. The resulting supernatant was pipetted into plastic tubes and was stored at -70°C until analysis. The kidney was used for determination of Superoxide Dismutase and Glutathione Peroxidase contain.
GPx assay methods - the Glutathione Peroxidase (GPx) activities were measured by RANSEL kit Glutathione Peroxidase by RANDOX company. After preparation of individual reagents and calibration Glutathione Peroxidase activity was measured spectrophotometrically at 340 nm and temperature of 37 °C. There was valuated the concentration of Glutathione Peroxidase from the calibration curve of the standard solution absorbance. The results of Glutathione Peroxidase concentration (U/l) were calculated as 8412*Δ340nm/minute. Subsequently the results were converted to U of GPx in 1 mg of total protein.
SOD assay methods - the Superoxide Dismutase (SOD) activities were measured by RANSOD kit Superoxide Dismutase, Diluent and Control by RANDOX company. After preparation of individual reagents and calibration Superoxide Dismutase activity was measured spectrophotometrically at 505 nm and temperature of 37 °C. There was valuated the concentration of Superoxide Dismutase from the calibration curve of the standard solution absorbance. The results of Superoxide Dismutase concentration were measured as SOD units U/g muscles. Subsequently the results were converted to U of SOD in 1 mg of total protein.

RESULTS AND DISCUSSION

The GPx analysis of kidney tissues showed significant differences ($p < 0.05$) among almost all monitored groups after 14 days of amygdalin administration. We observed significant differences between GPx activity in the control group and the experimental groups E1 and E2, where amygdalin was administered *i.m.* The significant difference was also observed between the control group and the experimental E4 group, where amygdalin was administered to the animals *per os* with a content of 300 mg mg/kg b.w. The significant difference was observed among almost all experimental groups except for experimental group E1 and E4 ($p > 0.05$). The activity of GPx is shown in Table 1.

Table 1 The activity of Glutathione Peroxidase in kidney tissue of rabbits (U of GPx/mg of protein)

	C group	E1 group	E2 group	E3 group	E4 group
$\bar{x} \pm SD$	30,08±2,6^{a}	72,26±15,01^{b}	123,6±16,55^{c}	36,88±13,12^{a,d}	82,57±14,19^{b,d}
v_k	8,56	20,78	13,39	35,59	17,19

Legend: C group – control group without amygdalin, experimental E1 group – *i.m.* application of amygdalin with concentration 0,6 mg.kg^{-1} b. w., experimental E2 group - *i.m.* application of amygdalin with concentration 3,0 mg/kg b. w., experimental E3 group – rabbits fed by crushed apricot seeds at dose 60 mg/kg b. w., experimental E4 group – rabbits fed by crushed apricot seeds at dose 300 mg/kg b. w.

We observed differences among SOD activity too. The significant differences ($p < 0.05$) were found out between the control group and experimental groups E2, where amygdalin was administered 3.0 mg/kg b. w. *i.m.* and between the control group and the experimental E4 group, where amygdalin was administered to the animals *per os* with a content of 300 mg mg/kg b. w. We found out significant differences ($p < 0.05$) between experimental groups E1:E2 (amygdalin was applicated 0.6 and 3.0 mg/kg b. w. *i.m.*) and between experimental group E1 (0,6 mg/kg b. w. *i.m.*) and experimental group E4 (300 mg/kg b. w. *per os*). We didn´t found out significant differences among other groups ($p > 0.05$). The activity of SOD is shown in Table 2.

Table 2 The activity of Superoxide Dismutase in kidney tissue of rabbits (U of SOD/mg of protein)

	C group	E1 group	E2 group	E3 group	E4 group
$\bar{x} \pm SD$	143,48±5,12^{a}	156,65±22,64^{a}	258,56±46,26^{b,c}	204,25±64,59^{a,c,d}	256,46±60,83^{b,d}
v_k	3,57	14,45	17,89	31,62	23,72

Legend: C group – control group without amygdalin, experimental E1 group – *i.m.* application of amygdalin with concentration 0,6 mg.kg^{-1} b. w., experimental E2 group - *i.m.* application of amygdalin with concentration 3,0 mg/kg b. w., experimental E3 group – rabbits fed by crushed apricot seeds at dose 60 mg/kg b. w., experimental E4 group – rabbits fed by crushed apricot seeds at dose 300 mg/kg b. w.

Kováčiková *et al.* (2019) conducted a study of the effect of amygdalin application on SOD and GPx activity in rabbits for 14 days. A significant ($p < 0.05$) change was detected in blood between the experimental group (application of amygdalin 300 mg/kg b. w.) and the control group (without application of amygdalin, during the experiment. Superoxide Dismutase activity in the experimental group significantly decreased (102.3 U/ml) compared to the control (117.4 U/ml) and the same tendency was observed in the second experimental group E3 with the application of amygdalin *per os* 60 mg/kg b. w. (111 .1 U/ml), however, this decrease was non-significant compared to the control group ($p > 0.05$). Activity of Glutathione Peroxidase was non-significantly but considerably higher in all experimental groups (E1, E2, E3, and E4) in comparison to control group.
Dawod and Ahmed (2021) observed significant elevating GPx in serum and SOD in hepatic tissue in groups rats. Animals were intake dose with 3 and 6 g/kg of apricot kernel powder. Authors concluded that administration with 6g/kg apricot kernel powder may increase risk of liver cell injury, 3g/kg apricot kernel powder was less effect and 1.5 g/kg apricot kernel powder shows no alteration in compared to control.
Some authors show (Newton et al., 1981) when amygdalin, a cyanogenic glycoside, is given orally or intravenously to rats, significant amounts of cyanide are released. The amount of cyanide liberated following oral administration is dependent in part on the bacterial flora of the gut and can be suppressed by antibiotic pre-treatment of the animals.
This can be one reason why results of experiments are different.
Newton *et al.*, (1981) warns according to their studies that if amygdalin is taken chronically, it will produce neurologic damage in humans similar to that seen in persons suffering from tropical ataxic neuropathy, a disorder attributed to chronic cyanide exposure. For their experiment they used clinically healthy animals.
The effects of amygdalin have been observed in many experiments. Authors investigated influence of amygdalin application on many dieses. **Guo *et al.* (2013)** found out that administration of amygdalin to rats alleviated the renal injury on the 21st day.
SOD levels in kidney tissues were all effectively reduced in experiment on high glucose-cultured renal cell HBZY-1 in vitro in rats by **Chen *et al.* (2021)**.

Many authors studied the protective effects of combination atorvastatin and amygdalin in a rat endometriosis model. GSH, SOD, GPx, and catalase levels increased by > 40%. The study suggested that combined treatment consisting of atorvastatin and amygdalin attenuates endometriosis (**Hu *et al.*, 2019**).

Kung *et al.*, (2021) presented study to explore whether amygdalin could ameliorate angiotensin induced cardiomyopathies. Additionally, oxidative stress related proteins including SOD-2, and GPX-4, were markedly increased following amygdalin treatment.

According to **Ismy *et al.* (2021)** one of the primary antioxidants that plays a role in warding off oxidative stress is Superoxide Dismutase (SOD) **(Ismy *et al.*, 2021**). Study of **Moslehi *et al.* (2019)** revealed that amygdalin pre-treatment significantly decreased malondialdehyde (MDA) concentration and increased antioxidant enzyme levels such as Superoxide Dismutase (SOD) and catalase (CAT). In the experiment tunicamycin (TM) was served that indicated endoplasmic reticulum (ER) stress in the liver tissue **(Moslehi *et al.*, 2019)**.

Autooxidation results in the formation of hydrogen peroxide, which inactivates SOD in diabetic patients. Decreased SOD activity may be caused by the accumulation of hydrogen peroxide. In addition, a high glucose condition can cause inactivation of this enzyme by its glycation. GPx is a relatively stable enzyme under normal conditions, while it can be inactivated under conditions of severe oxidative stress. In addition, hyperglycemia and overproduction of ROS suppress the endogenous antioxidant system and expose cells to damage due to oxidative stress (**Dworzański *et al.*, 2020**).

There were also conducted clinical trials were on healthy volunteers, where the effect of amygdalin was monitored (**Esra *et al.*, 2020**).

Wang *et al*. (2021) investigated the effect of amygdalin on liver fibrosis in rat model conditions. They found that assessing the drug efficacy based on this model, the different amygdalin doses significantly increased the SOD activity levels in the serum and liver tissues of the treatment groups, compared with those in the model group.

CONCLUSION

Our study provides information on the effect of amygdalin on the activity of sod and GPx in the kidneys of male California meat rabbit line P91 for 14 days of application. We found that administration of amygdalin intramuscularly and *per os* caused an increase in sod and GPx activity in kidney tissues of rabbits. Amygdalin contains a cyanogenic glycoside, which can have an adverse effect on the body. Since there are a few experiments that monitor the effect of amygdalin on tissues, we recommend continuing in the experiments.

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