Study of the Antioxidant effect of the total Hydroalcohol Extract obtained from the Vegetable Product *Satureja caerulea herba* in Experimentally Induced Tegumntary Burns in Wistar Rats

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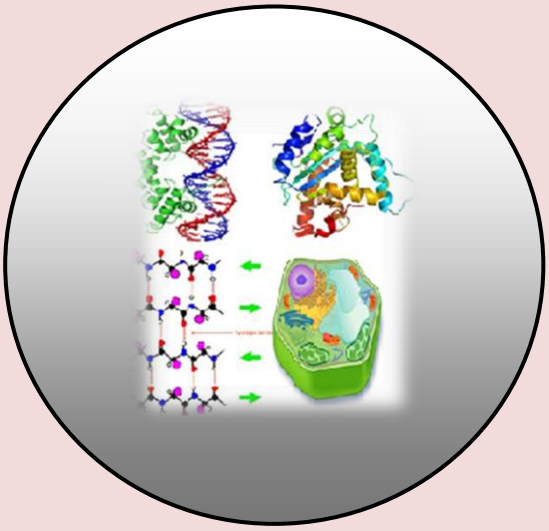

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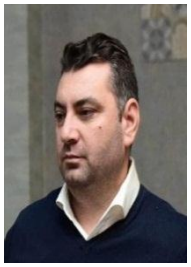

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Study of the Antioxidant effect of the total Hydroalcohol Extract obtained from the Vegetable Product *Satureja caerulea herba* in Experimentally Induced Tegumntary Burns in Wistar Rats

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ABSTRACT

In the last decade, the use of compounds derived from plants for therapeutic purposes in different pathologies in humans or animals, has gained popularity and has entered an exponential growth segment especially among the population in rural areas, but not only. The use of plant products in the treatment or prevention of diseases in animals and humans has become a priority, for both practitioners and especially for the scientific community. The experimental research aims to demonstrate the antioxidant action of the total hydroalcoholic extract obtained from the plant product Satureja caerulea herba in the skin burns experimentally induced in laboratory rats. From the experimental data obtained, after five days of local tegumental application of the phyto-product (total vegetable extract of Satureja caerulea Janka 10% in medical petroleum jelly), an adaptation of the defensive antioxidant defense system appears and a limitation of the effects, associated with the phenomenon of oxidative stress.

Keywords; Oxidative Stress; Satureja caerulea and Antioxidative Activity.

INTRODUCTION

Demonstrating that, active principles from plants could act as therapeutic potentials against different human or veterinary pathologies, has made plant products an indispensable asset in the prevention or treatment of human or veterinary pathologies (Ogbonnia et al., 2008, Steve et al., 2009, Assaei et al., 2015, Fereshteh et al., 2016). Herbal products with therapeutic activities remain the main source of biologically active principles. Phytomedication is the most common form of alternative medicine and is used by about 60% of the world's population, in both economically developed countries and the ones that are still in the development process, where modern drugs and functional food supplements from natural sources are predominantly used (Ogbonnia et al., 2008, Rickert et al., 1999). The use of plant phytocomplexes by traditional practitioners in the treatment of diseases is gaining more and more popularity and has entered a segment of exponential growth, especially among the population from rural areas in developing countries.

The popularity of phytomedication could be based on its therapeutic advantages, personal beliefs or the cheap cost of medication. Another way to promote phytotherapeutic products is to associate pharmacological chemical products with quantifiable adverse effects on the medical line, while phytopharmaceuticals are free of adverse or toxic effects to the body, which according to a scientific approach is not always correct because many active principles may exhibit toxicity to organisms (Ogbonnia et al., 2010, Oluyemi, 2007). With all these quantifiable benefits from the medical, financial and socio-cultural point of view, phytotherapeutic preparations can be contaminated with different chemical compounds used in phytosanitary treatments for crop pests or storage pests. Another danger of contamination is first of all the improper way of storing the plants and second of all but not less important the technology for obtaining biologically active fractions from plants. Following the technologies of extraction, separation, purification and encapsulation of the phytopharmaceuticals, residues can result that can negatively affect the health of the user. The presence of any of the sources of contamination represents a potential risk to the health of the phytomedication followers. Increased morbidity and mortality associated with the use of plants in different commercial forms has attracted increased attention in recent years (Bandaranayake, 2006). Phytotherapeutic preparations can give different forms of clinical toxicity, from mild to severe forms that can endanger the patient's lives. In most cases, the clinical toxic effect is represented by mixtures from different morpho-anatomical parts of plants belonging to different taxons. In most cases, the active principles in plants are not known for behavioral phytochemistry and there is the possibility of their negative association. The quality and safety criteria for herbal medicines are based on the quality and provenance of the raw materials used for such preparations. Phytotherapeutic products can be used in the treatment or as an adjuvant in a variety of pathologies (Pieme et al., 2006). Herbal medicines can also be administered over a long period of time without strict monitoring or dose adjustment to avoid cumulative toxic effects during prolonged treatment. In order to avoid the possible toxic effects at the liver and kidney level that may occur as a result of long-term ingestion of plant products, a clinician is required to monitor throughout the phytotherapeutic treatment (Tedong et al., 2007). Practically speaking, modern medicine has developed using traditional medicine and complementary therapies (Patwardhan, 2004). Administration of a naturally occurring biologically active substance or chemical synthesis for various purposes including the experimental one in an in vitro or in vivo biological system may immediately induce a series of reactions and an undesirable response immediately or over a period of time. Toxicity tests commonly performed in pharmaceutical practice when a new pharmacological compound is placed on the market are related to acute, subacute and chronic toxicity. Acute toxicity is involved in estimating the LD^{50} dose that has been shown to be lethal (causing death) in 50% of the animals in the experimental study. Determining the degree of toxicity by acute oral administration is usually an initial screening step in evaluating the toxic characteristics of all compounds regardless of nature. Acute toxicity is produced after administration of a single dose or multiple doses over a maximum of 24 hours, up to a limit of 2000 mg / Kg. The purpose of acute toxicity studies is to identify the dose that induces major and quantifiable adverse effects and an estimate of the minimum dose that causes mortality (Robinson et al., 2007). As we mentioned above, the major obstacle to the administration of traditional herbal preparations is the lack of experimental and clinical scientific data in support of a better understanding of the efficacy and safety of traditional medicines. In this case it is necessary to determine the degree of phytotoxicity and the biologically active doses that do not induce the occurrence of negative effects of any intensity. Skin burns are the most common form of skin damage in adults, especially children. The tegument is a simple structure from a histological point of view but extremely complex from a functional point of view. The tegument includes in its structure both vascular elements, the immune system but also a nervous component responsible for the two-way communication between the tegument cells and the nervous system. Neurophysiology has come a long way, today we are talking about a branch of neurophysiology called "cutaneous nerve science". As we have shown in the short presentation the tegument is a dynamic structure with vital importance being involved both in the protection of the organism but also in the neuro-metabolic and physiological regulation of the main systems, with experimental data regarding the above mentions. The use of phytotherapies in the treatment or prevention of animal diseases and humans has become a priority for both practitioners and especially for the scientific community.

The purpose of the research is the demonstration of the antioxidant action, of the total hydroalcoholic extract obtained from the plant product *Satureja caerulea* herb in the tegumentary burns experimentally induced in laboratory rats. The experimental research is part of a complex study where in addition to the topics presented, other aspects have been analyzed which includes the degree of phytotoxicity and the beneficial effect of the extract in moderating the effects before the bone integration of the dental implant. These studies will be published later. Genus *Satureja sp.* groups aromatic plants whose active principles can have varied effects from antioxidant, hepatoprotective, antibacterial, analgesic and anti-inflammatory to the effect of preventing morphine tolerance (Hajhashemi et al., 2012, Khaledi et al., 2020, Assaei et al., 2014, Dunkic, 2014, Saeeed Esmaeili et al., 2018). Based on the experimental data from the specialized literature but also on the treatment method of thematic burns, which includes a combination of analgesics, anti-inflammatories and antibacterials, we have established the experimental purpose and model for the present study.

MATERIALS AND METHODS

Plant Material and Preparation of Extracts

Satureja caerulea is a plant that grows in the spontaneous flora of temperate regions. In Romania it is found only near the Dobrogea region, being spread in the protected areas of Murfatlar region (Fântâniţa forest), Agigea, but also in Babadag forest region (Tulcea county). For the experimental study the plant was harvested from the Murfatlar area (Fântâniţa forest), outside the protected area between August 26-28, 2013. The aerial parts of the species were sorted and dried for 3 weeks, in an well ventilated room.

Extract Preparation. The aerial part of the plant was macerated in EtOH: H_2O (7: 3) for two days. The extract was then filtered and dried to dryness by removing the ethanol and water under pressure and in vacuum. 10 g of Extract from the aerial parts of the species *Satureja caerulea L.* from the extract obtained were dispersed in 90 g of Vaseline for experimental use on laboratory animals.

Animals Experimental Model

The animals used in our experimental model were 18 rats Wistar albino line, males 14 weeks of age and weighing 180-200g, in standard enclosures, with an air temperature of 22 ± 2, with the cycle 12 hour light / night with access to food and water ad libitum. Each experimental batch consisted of 6 animals. The experiments were carried out in accordance with the current norms regarding the protection of laboratory animals**.** The experiment was conducted in 2014.

Our experimental model is made up of three lots whose characteristics are:

- Male control group (AM) - the animals in this group were anesthetized and under experimental conditions a thermal burn was induced on a circular tegument beach with a radius of 1.5 cm. The animals in this group served as reference for the experimental groups.
- Male experimental group (AV) - the animals in this group was anesthetized and under experimental conditions a thermal burn was induced on a circular tegument beach with a radius of 1.5 cm. The animals in this group were administered the drugs for five days over the greasy beach.

Male experimental groups (AEx) - the animals in this group were anesthetized and under experimental conditions a thermal burn was induced on a circular tegument beach with a radius of 1.5 cm. The animals in this group were administered for five days over the burning beach the total vegetable extract from *Satureja caerulea* 10% in Vaseline 10 ± 2g, in a uniform layer.

After the five days of experiment, samples were taken under anesthesia, from the tegumentary area subjected to the thermal process of burn induction.

The parameters followed were: superoxide dismutase (SOD) and catalase (CAT) activity and low glutathione (GSH) tissue level. The enzymatic activity of superoxide dismutase was determined using the method described by Winterbourne (1979). The biochemical analysis method is a colorimetric method and the enzymatic activity is related to mg of protein. The determination of total proteins was done according to the method described by Lowry (1951). The determination of the enzymatic activity of catalase was done according to the method described by Beers and Sizer (1952). The biochemical analysis method is a kinetic method and the enzymatic activity is related to mg protein. GSH were analyzed in the tissue, using spectrophotometric determination, previously described by Beutler (1984).

Statistical Analysis
Data were processed in the program Origin Pro 75. The significance threshold was set at p≤0.05.

RESULTS AND DISCUSSION

The skin has an increased metabolic activity and as a result of the metabolic processes, final or intermediate products are obtained which are eliminated outside the body by specific means where they form the so-called biological aura specific to each individual and influenced by the sex, age and physiopathological state of the individual (Branza et al., 2014). As a result of the metabolic processes, free radicals of oxygen result (Urso and Clarkson, 2003). Free radicals are transient invasive chemical species of high chemical complexity, containing one or more odd electrons. Increasing the level of free radicals above the cell endurance limit leads to the installation of oxidative stress phenomenon with negative repercussions on the cell, a phenomenon that can lead to major cytolysis. Aerobic cells have developed a series of mechanisms for regulating the basal level of free radicals during evolution. The cellular mechanisms of regulation and defense against the actions of free radicals are represented by the enzymatic mechanisms having as representatives the enzymes superoxide dismutase, glutathione peroxidase and catalase and the non-enzymatic mechanisms represented by: vitamin A, C, E, reduced glutathione, α-lipoic acid, β -carotene, uric acid, bilirubin, coenzyme Q10, bilirubin but also bioactive principles in plants with antioxidant effect. In addition to the mentioned antioxidant mechanisms, cells also have other defense mechanisms against the action of the free radicals of oxygen. The negative effects of oxygen free radicals can be biochemically coupled and their action target is represented by all cell biomolecules, including information molecules (RNA and DNA). There is a balance between the antioxidant component and the level of free radicals. Altering the balance between free radicals versus antioxidants in favor of the first form, was first defined in 1985 as oxidative stress (Sies, 1985).

Table 1. Superoxide dismutase and catalase activity as well as reduced glutathione tissue level in the control group (AM) and experimental groups (AV and AEx).

		SOD (U / mg protein)	CAT (U / mg protein)	GSH (mcg / mg protein)
Male control group (AM)	X ± ES	3.46 ± 0.15	1.12 ± 0.24	4.27 ± 0.34
	n	6	6	6
Male experimental group (AV)	X ± ES	1.56 ± 0.23	0.60 ± 0.13	2.67 ± 0.17
	n	6	6	6
	t	3.54	4.54	6.54
	p≤	0.01	0.05	0.01
Male experimental group (AEx)	X ± ES	2.65 ± 0.21	1.27 ± 0.24	6.53 ± 0.25
	n	6	6	6
	t	-	-	5.54
	p≤	NS	NS	0.001

X±ES = mean± standard error; n = the number of individual samples that represented the arithmetic mean at the end; t = the value of the "t" test taken by the student; p = the threshold of significance established on the basis of the "t" value; NS = insignificant change.

Superoxide dismutase is an inducible enzyme that is dependent on biosynthesis and functionally high levels of superoxide radicals. In the case of our experimental model (see table 1.), the statistically significant enzymatic activity decreased after the five days of experiment in the experimental group (AV) compared to the reference group (AM), which indicates an increase of the level of superoxide radicals above the cell reference limit and the inability of the enzyme to catalyze the transformation of superoxide radicals into hydrogen peroxide radicals. In the case of the experimental group (AEx), the five days of phytotherapeutic treatment did not lead to statistically significant changes in the enzymatic activity of the superoxide dismutase compared to the reference control group (AM).

Catalase is an inducible enzyme that uses hydrogen peroxide as a substrate, a radical generated following the superoxide radical dismutation reaction, a reaction that occurs spontaneously or under the catalytic action of superoxide dismutase. Regarding the enzymatic activity of the catalase, in the case of the experimental model, the biochemical dynamics indicates a statistically significant enzymatic activity at the experimental group (AV) and statistically insignificant at the experimental group (AEx) compared with the reference control group (AM) (see table 1). This biochemical dynamics denotes an increased level of hydrogen peroxide radicals in the experimental group (AV), probably caused by a prolonged inflammatory state associated with an increase in the local metabolic rate. Reduced glutathione is a non-enzymatic antioxidant that can fulfill both the role of enzyme activator and the direct antioxidant role. In the case of our experimental model, the five days of experiment were sufficient to induce a statistically significant decrease in the tissue level of glutathione reduced in the experimental group (AV) compared to the reference control group (AM), this indicates an increased level of concentration of free radicals of oxygen at the tegumentary level. After the five days of phytotherapeutic treatment, we observe in the case of the experimental group (AEx) a significant increase in the tissue level of glutathione compared to the control control group (AM). This indicates an adaptation of the antioxidant system and avoiding the installation of the oxidative stress phenomenon with serious repercussions on the cell physiology.

CONCLUSION

The biochemical dynamics of the parameters of oxidative stress indicate that in our experimental model after five days of local tegumentary application of the phyto-product there is an adaptation of the defensive antioxidant defense system and a limitation of the effects associated with the phenomenon of oxidative stress. This adaptation is probably the consequence of the content of active antioxidant principles, the active principles responsible for the marked antioxidant activity determined in our experimental model.

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