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Impact of acute and chronic treatment on the production of prostaglandin E_2 in Wistar rats with nicotine extracts from oriental tobacco varieties

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***Abstract*:** During 2011 and 2012, on the experienced field of Scientific Tobacco Institute in Prilep experimental plots were set with oriental tobacco type Prilep (PP 66), type Jaka (JK-125/3) and type Basma.
Nicotiana tabacum L. which is the subject of the biochemical analysis is an important crop spread widely in the world. Nicotine, is the most important alkaloid in the tobacco plants, which has a big influence in the function of the organs in the living organisms.
Experimental part concerns determination of the effect of nicotine extracts from different oriental tobacco varieties in the production of prostaglandin E_2 in white laboratory rats after acute and chronic treatment. This research is conducted in order to determine the influence on the function of prostaglandin E_2 in the plasma of the experimental animals using ELISA test.

Key words: *tobacco, nicotine, prostaglandin E_2, white laboratory rat, ELISA*

I. INTRODUCTION

Tobacco (Nicotiana tabacum L.) is a species of the Solanaceae family, genus Nicotiana. This plant originated in America and is cultivated from 5000-3000 b.c. . Tobacco (Nicotiana tabacum L.) as a crop in respect of the area of distribution is a leading plant in the world [1]. Diversity of its' cultivation is based on the relative resistance of this type to adverse environmental conditions and low productivity of the soil. Apart from these features, the cultivation of tobacco is based on its' widespread use in tobacco-manufacturing industry and the pharmaceutical industry, especially in recent times when research in this area are more intense [2]. Nicotine which is the most important pyridine alkaloid is a powerful neurotoxin. It is considered that nicotine in combination with enzymes present in the dried tobacco material creates dependence in smokers. Due to its' lipophilic nature, the alkaloid is readily absorbed by the cell membrane. Any way of taking the tobacco is resulting in absorption of nicotine in varying concentrations in the bloodstream of consumers and at the time, development of dependency. New research in the field of plant physiology and biochemistry, which are based on the biosynthesis of alkaloids further increases the importance of these components and justifies the attention that is given [3].

Prostaglandins represent biological mediators arising from arachidonic acid, the so-called cyclooxygenase flow. They are synthesized in different cells as a response to physiological and pathological stimulation, act locally and their effects may be expressed in low concentrations. They are actually hormones, although rarely classified as such. They have short half-lives and their excretion is rapidly inactivated. Their action may takes place in the cell in which it is generated (autocrine) or a local effect (paracrine) [4].

Prostaglandins have a wide range of activities. Causing dilatation or constriction of vascular smooth muscle cells, aggregation and disaggregation of platelets. Act on spinal neurons causing pain. Other effects include the movement of calcium, muscle contraction, hormone regulation and cell growth control. Some prostaglandins induce fever, probably by participating in the mechanisms of thermoregulation in the hypothalamus and also have a role in inducing inflammation [4, 5].

In tissues where there is inflammation, there are inflammatory mediators that occur in response to inflammation. One of these mediators is PGE2.

II. MATERIALS AND METHODS

Object of analysis of this research is determining the impact of nicotine extracts of the most exploited types of tobacco in the Republic of Macedonia on the production of prostaglandin E_2 in white laboratory rat on a one-day, five-day and ten-day treatment.

2.1.Experimental design. The experimental animals used in this research represent white female laboratory rats of the genus Wistar. The animals were obtained from the animal facility of the Department of Physiology and Biochemistry of the Faculty of Natural Sciences and Mathematics, Skopje, Republic of Macedonia.The total number of animals used was n=76. They were divided into 18 groups of 4 animals that succumb to the acute and chronic treatment with aqueous extracts of nicotine from 3 oriental varieties of tobacco (Prilep, PP 66; Jaka, Jk-125/3; Basma) from two vintages, 2011 and 2012.
C-control group, n=4
Group 1 (one day treatment) , n=24
Group 2 (five day treatment), n=24
Group 3 (ten day treatment), n=24
The nicotine extracts were administered subcutaneously (s.c.) in amount of 200 μL every 0 hours, 6 hours and 24 hours.
2.1.1.After one-day treatment (24 hours) the first 6 groups of animals were sacrificed, plasma was collected and freezed at a temperature of -80 ° C. The other groups of animals were treated in the next 4 and 9 days. After the five day treatment and ten day treatment, the following 12 groups of animals were sacrificed. During the treatment was also observed the behavior of the experimental animals.
2.2.The concentration of prostaglandin E_2 in the plasma of white laboratory rats was quantitatively determined by using the ELISA test. For this purpose was used Cayman PGE_2 Express EIA whale that has been validated for use on samples of tissue culture, plasma, and urine. The analysis is based on the competition between PGE_2 and PGE_2 - acetylcholinesterase conjugate (PGE_2 tracer) for a number of PGE_2 monoclonal antibody.
Collected plasma samples undergoing on purification and homogenization immediately before use to remove all antibodies that can interfere in the further analysis. Purification of the samples and thew elution of PGE_2 were carried out using SPE cartridges (C-18). Resuspending of the eluated sample was carried out by adding 500 μL EIA buffer. The sample was ready for use.
2.3.All samples for analysis, including the standard solutions with precisely known concentrations of PGE_2 were placed on the plate for analysis and after a certain period (60min) absorbance was read at a wavelength of 420nm on a microplate reader.
2.4. **.**Statistical analysis. ANOVA was used for determination of statistical significance. A $p<0.05$ value was considered significant.

III. RESULTS AND DISCUSSION

The survey results give a clear picture of the impact of nicotine on the production of PGE_2 in white laboratory rat after acute and chronic treatment with aqueous extracts of the most exploited oriental varieties of tobacco in the country.

During the survey was noted the behavior of experimental animals, whereas it was concluded that those animals which were exposed to the one-day treatment had no significant change in behavior, but those experimental animals that were subjected to a five-day and ten-day treatment showed significantly hyperactive and aggressive behavior.

Nicotine intoxication in white laboratory rat (Wistar-rats) after several days showed differences in the production of prostaglandin E2 in blood plasma of different varieties of tobacco collecting during two growing seasons (2011/2012). The results of production (release) of PGE2 in blood plasma after intoxication with nicotine extracts from various varieties of tobacco in an initial (control) period and extended application period vary. Differences exist within it and the varieties within the crop. What will be the response of the body depends on the type and age of the body, the concentration of the applied agent, duration of application, microclimate factors and many other factors. The results will be disseminated in the text below.

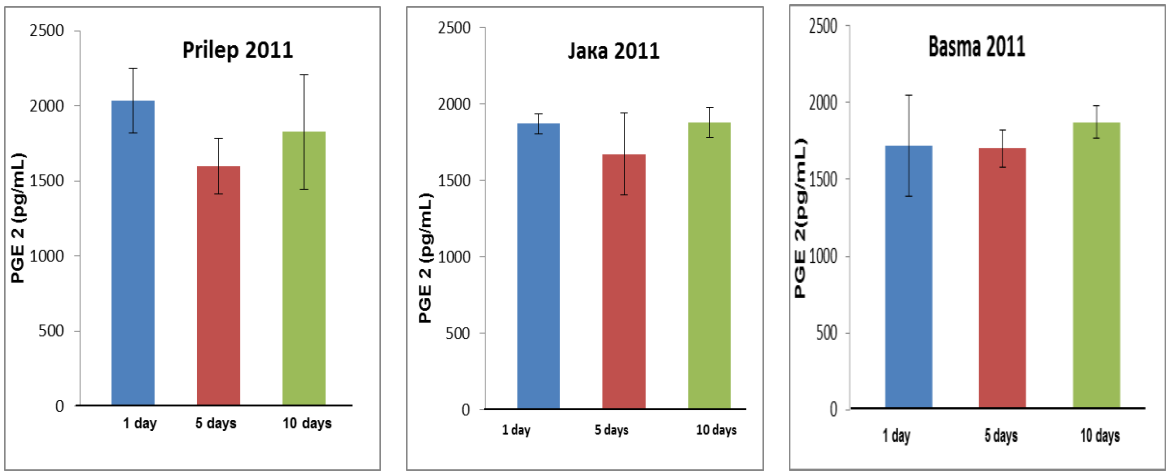

Figure 1 Production of prostaglandin E2 (PGE2) in blood plasma (pg / ml) in white laboratory rat after one day, five days and ten days treatment with nicotine (200µl) of three varieties of tobacco (2011)

If a review of histogramic display (Fig. 1) in both varieties of tobacco (Prilep and Jaka) after one-day treatment is a tendency to initially increase the concentration of PGE2. These values (pg / ml) are in the limits of 2034,1 ± 213,17 in the first day to five days after (1597,3 ± 183,90) noted a tendency for sharp reduction in the production of prostaglandin and again increasing the values (1825,2 ± 381,51) in the tenth day. It suggests that the continued nicotine intoxication in the body of the white laboratory rat causes changes in the early reaches a certain level of adaptation to the changes to the fifth day after the production of eicosanoids this decline. Body this stressor factor experiences by establishing a so-called equilibrium comprises (lag phase) to immediately (after 10 days) appeared to sharply raise the concentration of PGE2 which probably would go toward much higher values depending on the prolonged period of intoxication with the nicotine. Increased values of production of PGE2 correlate with external behavior intensively followed with great anxiety and movement in space. Such symptoms of behavior observed in the first few days and treatments were made after 0 hours, 6 hours and 24 hours. Similar values were obtained with the treatment from 2012 of the same kind where differences between the results obtained from the first year slightly changed and are in the limits (pg / ml) of 1804,8 ± 229,16 in the first day, 1772, 90 ± 336,50 in the fifth day and 1896,90 ± 117,99 per ten-day treatment.
Similar results with small deviations are found among the variety Jaka in respect of the results obtained during the entire treatment. The single treatment with nicotine does not show cytotoxic continuous changes that are sustainable long time and does not cause morphological manifestations and certain enzyme activities and transformations of prostaglandins in certain tissues and organs [6].
Following histogramic views of both varieties and the results of the liberated PGE_2, observed nearly identical changes. In this variety they are in the limits of 1871,40 ± 64,57 in the first day, 1673,00 ± 267,01 in the fifth day and 1880,40 ± 100,20 in the tenth day (2011). The following 2012 was concluded directly proportional dependence in relation nicotine - PGE2. As the period of continuous intoxication and release of PGE2 was more intense. Growth in production ranges from 1506,7 ± 397,82; 1790,7 ± 233,65; 2109,8 ± 198,97 pg / ml. (Table 1)

Table 1 Statistical review of the produced PGE2 (pg / ml) after several days of treatment with nicotine from three varieties of tobacco during two growing seasons (2011/2012)

Tobacco variety	Harvest	1 day	5 day	10 day
Prilep 66	2011	2034,1±213,17	1597,3±183,90	1825,2±381,51
	2012	1804,8±229,16	1772,9±336,50	1896,9±117,99
Basma 82	2011	1717,5±329,79	1702,4±120,30	1872,8±104,05
	2012	1660,1±204,71	1764,7±44,37	1875,3±154,,27
Jaka 125/3	2011	1871,4±64,57	1673±267,01	1880,4±100,20
	2012	1506,7±397,82	1790,7±233,65	2109,8±198,97

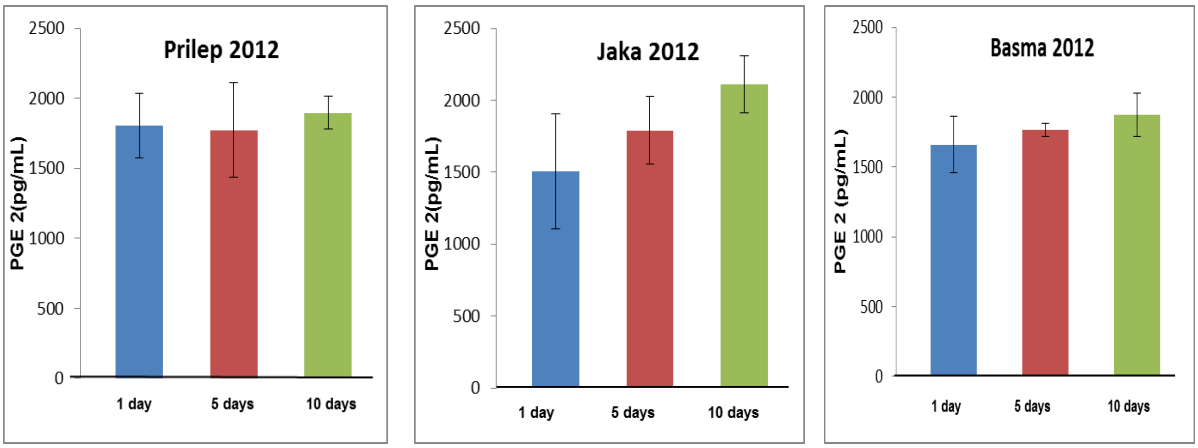

Figure 2 Production of prostaglandin E2 (PGE2) in blood plasma (pg / ml) in white laboratory rat after one day , five days and ten days treatment with nicotine (200µl) of three varietes of tobacco (2012)

Lower values of PGE2 in blood plasma resulted in the variety Basma. These values are almost identical in the nicotinic treatment of variety of both crops. And here is a slight increase in the production of PGE2 or followed by numerical values (Table 1) they are: 1717,5 ± 329,79; 1702,4 ± 120,30 and 1872,8 ± 104,05 (2011) and 1660,10 ± 204,71; 1764,70 ± 44,37 and 1875,30 ± 154,27 (2012). And in this variety was observed linear dependence (2012), which released prostaglandin is correlated with prolonged nicotine treatment (Fig.2) .

IV. CONCLUSION

Increased production of PGE2 is determined after acute and chronic treatment with nicotine extracts from different oriental tobacco varieties. This is corelated with the behavior of the Wistar rats. PGE2 is known lipid mediator who participates in the regulation of key responses in major human systems including the reproductive, gastrointestinal, neuroendocrine and immune system and its increased concentration affects the function of many organs in the body [7].

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