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Effect of Long-term Intake of Aspartame on the Hematological Parameters in Male and Female Albino Rats, and the Possible Ameliorative Effects of Garlic , Melatonin and Thymoquinone

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Abstract: Aspartame is a synthetic dipeptide, frequently used as nonnutritive sweetener in many worldwide countries. It is used as a feed additive in diet food products and diet drink. Aspartame is used by diabetics and people in different diets. There have been contradictory reports on the use of aspartame, that there are potential side effects associated with aspartame consumption on many organs in the human and experimental animals. This study investigates the long-term administration (six months) of aspartame on hematological parameter of male and female albino rats, and the possible role of garlic, melatonin and thymoquinone in ameliorating the pathological changes induced by aspartame. Rats were administrated with aspartame (40 mg/ kg/ body wt daily) for six months in drinking water. Also rats were provided with aspartame combined with garlic (10 mg/kg .body wt), with melatonin (10 mg/kg body wt.) and with thymoquinone (10 mg/kg body wt.) daily for six months.

Results showed that administration of aspartame caused a significant increase in the number of red blood corpuscles (RBCs) and a significant decrease of hemoglobin (Hb) concentration of both male and female rats .Also ,aspartame resulted in a significant decrease in the number of white blood cells (WBCs) and neutrophils .However ,a significant increase in platelets ,lymphocytes ,monocytes ,basophiles and eosinophils was observed in male and female rats after aspartame intake .

Administration of aspartame combined with garlic, with melatonin, with thymoquinone and with melatonin plus thymoquinone much reduced the toxic effects of aspartame on the above mentioned parameters. They normalized the values of the hematological parameters and restored them to the values similar to that of the control or near to it. These findings demonstrate that garlic, melatonin and thymoquinone, as antioxidants, ameliorate the toxic effect of aspartame on the blood parameters in male and female albino rats. This study concludes that aspartame (40 mg/kg.bw) intake for long-term (six months) may cause oxidative stress by altering the oxidant/antioxidant balance in the blood parameters and the supplementation of garlic , melatonin and thymoquinone, as antioxidants ameliorate the toxic effect of aspartame on blood parameters in male and female rats.

Keywords: Aspartame ,Melatonin,Thymoquinone.

1 Introduction

Aspartame (L-aspartyl - L-phenylalanine methyl ester) is widely consumed by diabetic humans and those who are under weight loss regime. It is added as sweetener to a large variety of food which is found in a low calorie beverages, desserts and table top sweeteners added to tea or coffee[1,2]. It is a synthetic dipeptide artificial sweetener formed by the reaction of L-aspartic acid with L-phenylalanine methyl ester [3] After oral administration of aspartame to humans and experimental animals, aspartame is rapidly and completely metabolized to aspartic acid, phenylalanine and methanol [4] .It is known that methanol is a substance which causes damage in the liver cells where it is oxidized to formaldehyde and finally to formate [5]. Thereby it leads to an elevation in NADH levels and the formation of superoxide anion which may result in a significant increase in lipid peroxidation [6].

Oral administration of aspartame at a higher dose (more than 40 mg/ kg/body wt. daily) and at a safe dose (equal or less than 40mg/ kg/body wt. daily) [7,8,9] leads to an increase of free radical production and induce oxidative stress in blood cells (Red blood corpuscles, neutrophils and lymphocytes) via altering the oxidant/ antioxidant balance. Oxidative stress in erythrocytes can lead to damage of erythrocytes membrane [10] ,consequently impair the main function of erythrocytes for transporting of hemoglobin which supply oxygen to all tissues of the body inducing erythrocyte aging1[11] and inflammation [12].Hemoglobin which functions as an oxidase, high pressure oxygen in circulation, membrane proteins and unsaturated fatty acids which can be oxygenated, these factors when combined with each other create a suitable environment for potentially harmful reactions for erythrocytes, then lead to premature, dysfunction and death of cells [13] White blood cells and their types (neutrophils and lymphocytes) are considered to be defense against the infection of bacteria and other harmful microorganisms play an important part in the production of active oxygen and its derivatives (hydrogen peroxide, hydroxyl group and single oxygen) by NADPH oxidase[14] .The active oxygen- producing granules inside neutrophils directly fuse to plasma membrane or form large endocytic vacuoles which bind to plasma membrane (Kobayashi *et al*., 1998). Consequently this causes release of active oxygen and its free radicals to destroy normal components of the body such as cells , tissues and metabolic pathways leading to various pathological changes [16,17].

Garlic (*Allium sativum L*.) among the oldest cultivated plants which are used as food and for medicinal applications. It is available as powder (tablets), as aged extracts (capsules, tablets and liquid) and as oils (capsules).It is known since long ago and has been used by the ancient Egyptians in the treatment and prevention of various diseases [18] .It is known as important food of the Mediterranean diet, since it is a rich source of several phytonutrients [19] . Garlic powder has been reported to have antioxidant properties and act as scavenger against free radicals [20].The antioxidant activity of the garlic components is due to the four main chemical classes, alanine, allyl cysteine, allyl disulfide and allicin [21] .Supplementation of aqueous garlic extract (125 mg/kg.b .wt.) for 28 days (3 times/ week) caused a significant increase of white blood cells (WBCs), number of RBCs ,hemoglobin content, while it led to a significant decrease in hematocrit value (PCV) in *Schistosma mansoni* infected mice compared with non-infected control group[22] ..These results indicate that garlic extract possesses antioxidant activity against the pathological changes in the blood parameters induced by the infected *S. mansoni* in mice.

Melatonin is secreted by major cells of pineal gland and its maximum secretion occurs during night [23] .It is a hormone synthesized from an essential amino acids tryptophan[24]. It acts as strong antioxidant and anti-inflammatory and can be considered as a novel cell protector in extra- pineal gland [25] .It protects heart ,liver, kidneys , stomach, lungs and skin from oxidative stress [26,27] .It acts as a free radical scavengers via enhancing the transcription of the antioxidant enzyme levels of various organs of the body [28]. Moreover, melatonin inhibits lipid peroxidation [29] .Melatonin normalized the pathological changes induced by heavy metals in the hematological parameters in albino rats [30]. Also, melatonin can decrease the toxic effect of many environmental chemicals which induce oxidative stress [31].

Thymoquinone is the main constituent of the volatile oil isolated from *Nigella sativa* seed which has various pharmacological actions [32]. Thymoquinone acts as antioxidants due to its free radical scavengers [33].It has been reported that thymoquinone showed a protective effect against natural and chemical toxins which induced pathological changes in the biochemical parameters of soft tissue such as liver and kidney [34] in the experimental animals. Also it acts as antioxidant against the toxic effects of toxic metals and chemicals on erythrocytes of mice [35[and on the hematological parameters in albino rats [30] .

Since the effects of aspartame on the hematological parameters have been given little attention, the present study aimed to explore the pathological changes induced by aspartame on the hematological parameters in albino rats. Also, the ameliorative effects of garlic, melatonin and thymoquinone (as antioxidants) were used to investigate their actions against oxidative stress exerted by aspartame, since little information about their ameliorative roles against toxic effect of aspartame on blood parameters in the experimental animals are available.

2 Materials and Methods

Animals: The experimental animals were healthy inbreed male and female albino rats (*Rattus rattus*) weighing approximately 160- 180 gm (8-10 weeks of age). The animals were obtained from the animal house of the medicine faculty, Assuit university. The total number of the experimental animals used in this study were 84 rats (42 males and 42 females). They were housed in stainless steel cages, six per each cage at room temperature and acclimated to laboratory conditions two weeks before experimentation, with 12-hr light and 12-hr dark exposure. They were allowed to have food and water *ad libitum* (standard rat food pellets).

Chemicals: Aspartame (L- aspartyl-L-phenylalanine methyl ester), garlic, melatonin (n- acetyi-5-methoxytryptamine) and thymoquinone (2 isopropyl-5-methyl- 1.4- benzoquinone) were purchased from Loba Cheme (India). All the chemicals used were of highly analytical grade.

Experimental protocol: After an acclimation period, the animals of each gender were divided randomly into seven groups (6/ group) and were treated with the treatments for six months. The first group was given drinking water and served as control group; the second group was given aspartame dissolved in distilled water in a dose of 40 mg/kg b.wt/ day; the third group was given garlic dissolved in distilled water at a dose of 50 mg/kg b.wt/ day; the fourth group was given aspartame and garlic respectively/ day; the fifth group was given aspartame and melatonin dissolved in distilled water at a dose of 10 mg/kg b.wt/ day respectively; the sixth group was given aspartame and thymoquinone dissolved in distilled water in a dose of 10 mg/kg b.wt/ day respectively , whereas the seventh group was given aspartame and melatonin plus thymoquinone/ day respectively. All groups were given the treatments morning, except melatonin was given between 09.00 and 10.00 pm. One day after the final treatments, the animals were euthanized by inhalation of chloroform. Blood was collected by cardiac puncture into EDTA sterilized sample tubes., Italy). The analyzed hematological parameters were red blood corpuscles (RBCs) count, hemoglobin volume (Hb), white blood cells (WBCs) count, platelets (PLT) count , neutrophils, eosinophils, basophils, monocytes and lymphocytes count (spectrophotometer, Cline Check Plus ,Italy)

Results: At the end of experimental period (six months), the ameliorative role of garlic, melatonin and thymoquinone against the toxic effect of long- term administration of aspartame on the hematological parameters were evaluated. All the results from various groups have been compared with the normal control group and aspartame treated group. All the animals survived till the entire course of the study without illness.

The results of the present study revealed a significant ($p< 0,05$) increase in the RBCs count in male and female rats, relative to the control group. In male and female rats, a significant ($p< 0.05$) decrease in the Hb concentration was noticed, after administration of aspartame (Table1 ; 2).

The administration of aspartame caused a significant ($p<0.05$) decrease in WBCs and neutrophils count, but it caused a significant ($p< 0.05$) increase in PLT ,eosinophils, basophiles, monocytes and lymphocytes counts in both male and female rats, relative to that of the control group (Table 1;2).

In comparison with aspartame treated group, each of garlic, melatonin, thymoquinone and of melatonin plus thymoquinone restored the values of the hematological parameters in male and female rats to the values either similar or near to the values of the control group. It should be noted that a pronounced protective effect on hematological parameters against the pathological changes of aspartame was observed after administration of melatonin plus thymoquinone. Also , thymoquinone has a positive effect on pathological changes induced by aspartame than that of melatonin. Moreover garlic has a pronounced role by decreasing the aspartame increased-parameters and raised the decreased aspartame parameters to the values similar or near to the value of the control group (Table 1;2).

Table 1 :- The influence of different treatments on the hematological parameters in male rats.

Parameter	control	aspartame	Garlic	Asp. +Garlic	Asp. +Mela.	Asp. +Thym.	Asp. + Mela. +Tthym.
RBCs [10^6/mm^3]	6.3±0.14	6.66±0.22*	6.11±0.12*○	5.98±0.098*○	6.38±0.15●○	6.51±0.34●●	6.13±0.12●○
HB [g/dl]	14±0.3	12.3±0.4*	14.2±0.2●○	14.3±0.2●○	14.2±0.4●○	14.1±0.4●○	14.4±0.1 *○
WBCs [10^3/mm^3]	7.266 ±796.6	4.6833 ±371*	7.233±771.1●○	7.200±303.3●○	7.0333±484.4●○	7.250±350.7●○	6.850±824.01●○
PLT [10^3/mm^3]	270.6±14.1	281±14.2*	270.8±9.2●○	275.8±12.4*●	280.8±12.4●●	271±11.8●●	268.06±6.9●●
Neutrophils [10^3/mm^3]	4.511 ±491.9	2.975±280.5*	4.493±480.2●○	3.643±726.2*○	3.291±663.6●●	3.553±457.2●○	3.366±177.9●○
Eosinophils [10^3/mm^3]	.165 ±4.47	.195 ± 13.57*	.170 ± 6.3●○	.177 ± 2.73●○	.169. ± 6.65●○	173 ± .7.745●○	.172 ± 9.95*○
Basophils [10^3/mm^3]	.0288 ± 0.98	.0322 ±2.25*	0291 ± .0.98●○	.0301 ± 1.6●○	.0263 ± 3.38●○	0293 ± .0.816●○	0288 ± .1.329●○
Monocytes [10^3/mm^3]	.383 ± 16.32	.416 ± 14.2*	.388 ± 14.7●○	.375 ± 15.16●○	.313 ± 9.83*○	.346 ± 47.6●○	.356 ± 32.04●○
Lymphocytes [10^3/mm^3]	21.247±68.8	43.467 ± 263.2*	21.45 ± 37.28●○	23.417 ± 241.2●○	22.50 ± 279.6●○	22.00 ± 83.7●○	24.00 ± 167.33*○

Table 2 : The influence of different treatments on the hematological parameters in female rats.

parameters	control	aspartame	Garlic	Asp. +Garlic	Asp. +Mela.	Asp. +Thym.	Asp. + Mela. + thym.
RBCs [10^6/mm^3]	5.2±0.2	6±0.1*	5.1±0.4●○	5.2±0.4●○	4.5±0.3●○	4.5±0.2●○	4.8±0.3●○
HB [g/dl]	10.5 ± 0.9	5.6 ± 0.2*	10.6 ± 0.9●○	10.4 ± 0.7●○	12.7 ± 0.5*○	12.5 ± 0.5*○	13.1 ± 1.1*○
WBCs [10^3/mm^3]	8.116±511.5	4.567±338.6 *	8.000±109.5 ●○	7.500±433.6●○	7.433±508.6*○	7.500±404.9*○	6.950±1677.8* ○
PLT [10^3/mm^3]	24.53±10.6	27.73±2.9*	24.53±8.7●○	25.42±18.3●○	25.15±10.2*○	24.58±10.4●○	24.75±11.4●○
Neutrophils [10^3/mm^3]	5.033±317.4	2.241±990.4 *	4.935±63.5● ○	4.738±348.9●○	4.467±206.5*○	4.933±564.5●○	4.717±147.2● ○
Eosinophils [10^3/mm^3]	.170 ± 4.92	.236± 49.3*	.171 ± 5.84●○	.175 ± 5.85●○	.177 ± 5●○	.179 ± 4.92●○	.181 ± 6.05*○
Basophils [10^3/mm^3]	.0315 ± 1.97	.0355 ± 1.37*	.0315 ± 2.26●○	.0326± 2.73●○	.0293 ± 1.33*○	.0296 ± 1.5●○	.0325 ± 2.25●●
Monocytes [10^3/mm^3]	.383 ±27.3	.425 ±21.67*	.385 ± 22.45●○	.365 ± 33.9●○	.326 ± 23.4*○	.324 ± 27.38*○	.340 ± 37.42●○
Lymphocytes [10^3/mm^3]	21.55 ± 39.37	46.667 ± 250.3*	21.833 ± 191.5●○	23.4.3 ± 173.97●○	22.267 ± 251.9●○	21.367 ± 117.7●○	2115 ± 92.5●○

The number of rats in each series were 6.

- ● P > 0.05(Non-significant).
- * P < 0.05 (Significant difference with respect to control group).

3 Discussions

The current study was designed to investigate the ameliorative effect of garlic ,melatonin ,thymoquinone and the combination of melatonin and thymoquinone against aspartame- induced blood cells toxicity in male and female albino rats.

Aspartame is well known as a synthetic chemical consists of approximately 50% phenylalanine, 40% aspartic acid and 10% methanol [36] .It has been established by the FDA and European food safety authority that the acceptable daily intake level of aspartame. is 40 and 50 mg/kg/ b.wt/ day. Small amounts of aspartame caused an increase in the methanol concentration in the blood stream. Methanol itself has low toxicity, however its metabolites are very toxic [6] .Methanol intoxication resulted in the formation of superoxide anions and lipid peroxidation [37]. Oral administration of aspartame for long duration creates the environment for potentially harmful effects on the blood cells [37].

A significant increase in RBCs count was observed in male and female rats after administration of aspartame for six months, compared with that of control. These results agree with the study of [38] who stated that administration of aspartame for 4 weeks caused a significant increase in RBCs count. However, oral administration of aspartame resulted in non- significant changes in RBCS count [39] .But, in contrast to our findings , it has been reported that aspartame led to a significant decrease in the RBCs count [40].

It has been reported that the increase in the RBCs count may be related to the ability of aspartame to induce proliferation of haemapoietic tissue to increase RBCs [41]. Also, it has been documented that aspartame could induce free radicals and thus induce cell damage by their severe cytotoxic effects, such as lipid peroxidation and protein

oxidation in the cell membrane which lead to alteration in the membrane integrity due to methanol exposure [9] which the metabolite of aspartame may induce generation of free radicals , thereby cause changes in the value of RBCs count.

As evaluated in the current study, aspartame administration is associated with a significant decrease in the blood hemoglobin. Similar results were obtained by [42]. However, research studies on the effect of aspartame on the hematological parameters in Wistar albino rats indicated that aspartame resulted in a significant increase in the hemoglobin levels [43]. In contrast to the above results, it has been demonstrated that aspartame, irrespective of its duration, did not cause any change in the hemoglobin levels in Wistar albino rats [8]. .In the study of [42], the decrease in the hemoglobin was associated with a greater decrease in the serum levels of iron and ferritin as well as increased levels of serum TIBC and UIBC. These changes may be related to the methanol intoxication which increased the production of free radicals levels in the blood cells [44]. So ,it can be concluded that aspartame disturb the oxidant/ antioxidant balance and induce oxidative stress which may led to the changes in the RBCs count and hemoglobin levels evaluated in the current study.

As demonstrated in the current study, garlic, melatonin and thymoquinone administration normalized the aspartame – intoxication in a way that they restored the RBCs count and hemoglobin levels to the values similar or near to that of the control. Oxidative stress is known to play an important role in the generation of free radicals in various kind of stresses [45,46] .So, the possible reason for restoring the normal values of RBCs and hemoglobin levels on administration of garlic may be due to the organosulfur content present in the garlic which acts as antioxidant preventing free radicals generation [47] against oxidative stress of aspartame on RBCS count and hemoglobin levels. Hence ,it can be concluded that the antioxidant activity of garlic may enhance the changes in RBCs count and hemoglobin by amelioration of the damage on the target organs .Melatonin and its metabolite proved their antioxidant activity and protective effects on oxidative stress, as expected to be found in living organisms wherever melatonin is produced, against toxic effects of many drugs in the biological organs [48]. Thymoquinone, according to several line of evidence , has been proved to have protective effect against toxic agents either natural or chemical toxins on different tissues of animal studies [49]. Also, it has been reported that thymoquinone has a strong antioxidant activity due to its free radices scavenging activity [33].So , it can be concluded that melatonin and thymoquinone , as antioxidants, may have ameliorative effect against aspartame intoxicatation on the RBCS and hemoglobin. Also, they might be protect the hemapiotic tissues against the damage excreted by aspartame .

As illustrated in the current study, administration of aspartame for six monthes resulted in a significant decrease in WBCs and neutrophils counts, whereas a significant increase in the platelets, eosinophils , basophiles, monocytes and lymphocytes were observed, in comparison with that of the the normal control group. Similar results were obtained by [8] who stated that long-term administration of aspartame caused a significant decrease in WBCS and neutrophils count, and significant increase in the lymphocyte . But , non- significant increase in eosinophils and monocytes count were documented. In consistent with our findings, [39] stated that aspartame caused a significant depletion in WBCs ,however, in contrast to our findings and to the above results it has been demonstrated that oral administration of aspartame caused a non- significant increase in WBCs, platelets, neutrophils, eosinophils, monocytes and lymphocytes count [38]. The decrease in WBCs count in aspartame-treated animals is due to redistribution of cells into damaged organs such as liver rather that loss of cells [50] . The decrease in neutrophils and the increase in lymphocytes can be attributed the opposite directions of both cells. The reduction in the neutrophils can be attributed to the margination of neutrophils [51] ,and this may be result from its abnormal distribution due to chemotaxis that causes retention of cells in several organs [8]. These changes may be due to the generation of high levels of free radicals which are reactive and interact with cellular macromolecules such as lipid membranes, carbohydrates , proteins and nucleic acids, thereby interfering with vital cellular function [52]. So, It can be concluded that the long-term administration of aspartame induced more oxidative stress which imbalance the homeostasis in the target organs. Also , these changes in the blood cells may be due to generations of high levels of free radicals via methanol metabolite of aspartame .

The antioxidant activity of garlic , melatonin and thymoquinone . was proved in this study on the oxidative stress induced by aspartame on WBCs, platelets, neutrophils, eosinophils, basophils, monocytes and lymphocytes in male and female albino rats it means that these antioxidants normalized the values of the above

blood parameter to the values similar to that of the normal control values. Furthermore , these antioxidants have ameliorative effects against aspartame toxicity.

The results of current study indicated that long-term intake of aspartame induced oxidative stress in the estimated blood parameters which lead to alterations in these cells. On the other hand, administration of garlic, melatonin and thymoquinone restored the alterations in the blood cells to their normal values in a way that these antioxidants have ameliorative effect against aspartame intoxication .

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