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Original Research Article

Effects of benzopyrene experimental intoxication and its treatment by star anise on hematological parameters in rats

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ABSTRACT
This study aimed to explore the effects of benzopyrene experimental intoxication and its treatment by star anise on hematological parameters in rats. 30 male white Swiss rats (Sprague Dawley), aged 3-4 months and weighing between 170-210 g. The rats were divided into six groups: Control (G1), B[a]P (G2), Star Anise 125 mg/kg bw (G3), Star Anise 120 mg/kg bw + B[a]P (G4), Star Anise 125 mg/kg bw + B[a]P (G5), and Star Anise 130 mg/kg bw + B[a]P (G6). Blood was collected by cardiac puncture at end of experiment by using tubes with EDTA and hematological parameters were estimated by using auto blood analyzer. Erythrogram didn't showed any significant differences between studied groups, while These results showed that exposure to benzopyrene significantly increased all measured WBC parameters, indicating an inflammatory and immunosuppressive response. The mean values of Mid, Gran, L, and total WBC counts in Benzo group were 3.93 ± 0.97, 12.26 ± 1.08, 10.01 ± 1.07, 25.46 ± 1.29, respectively. The mean values of Mid, Gran, L, and total WBC counts in treatment groups significantly decreased in comparison with Benzo group. They are 1.11 ± 0.01, 4.41 ± 0.52, 5.10 ± 0.92, and 10.27 ± 0.31, respectively. These results indicated that benzopyrene can induce an immune response with leukocytosis and a population increase in the absolute granulocyte and lymphocyte, those treatments can reduce these values, indicating the potential protective effects of them on benzopyrene immune toxicity. This results show that the treatment of rats with benzopyrene caused changes in a number of WBC parameters that reflects an immune inflammatory response and could also reflect immunosuppression without any effects on the erythrogram. Such changes were reduced by the treatment with Star anis showing higher efficacy, possibly indicating a role for these medicinal plants as protective agents against benzopyrene-induced immunotoxicity.
Keywords: Benzopyrene, star anise, WBC, RBC, Erythrogram)

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1. Introduction

The mechanisms of benzopyrene intoxication in toxicology and pharmacology and the promising treatments are highly important in many healthcare settings. Benzopyrene produces serious human health problems; for this reason, the status of the hematological parameters is often changed in patients with benzopyrene intoxication (1). It would be helpful for human health if natural remedies, such as star anise, produce

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some positive results on the treatment of benzopyrene intoxication. Investigation into the mechanisms of benzopyrene toxicity and the potentially promising role of star-anise could offer beneficial aspects for hematological parameter disorders in humans exposed to environmental pollutants(2).

Hematological parameters are an effective way to identify, describe, characterise and treat toxicity induced by benzopyrene intoxication. Some toxic effects of benzopyrene are oxidative stress, DNA damage and inflammation. An example of a hematological parameter is the red blood cell count that describes the number of red blood cells (3). Exposure to benzopyrene intoxication can affect Haematic parameters such as red blood cell count, haemoglobin, WBC, MCHC, MCV and MCH. Rats show an imbalance in the hematological parameters as an oxidative stress response to the genotoxic, haematotoxic and carcinogenic effects of cigarette smoke and benzopyrene toxicity. By identifying the changes in benzopyrene-induced hematological parameters in rats, we can determine the effect of a new treatment that can help reduce the deleterious effects of benzopyrene intoxication(4).

All these parameters are extremely important in indicating rats health and biological responses at physiological level due to benzopyrene intoxication. It was determined that chronic exposure to benzopyrene induced alteration in the red blood cell count, hemoglobin and white blood cell counts of rats (5). Decrease in the red blood cell count and haemoglobin levels are evidence that benzopyrene intoxication might induce anemia in exposed animals, which can lead to defects in physiological functions of the body, while impacts on white blood cell count indicate that benzopyrene intoxication can alter immune response against blood cell production which in turn may increase the susceptible for respiratory tract infections(6).

Among the metabolic changes observed in rat blood, the benzopyrene intoxication can cause significant changes in haematological parameters, such as red blood cell count, hemoglobin level, and white blood cells, which may warrant the treatment of plant products as a source of free radical scavenger or natural chemo-preventive agent to counteract the toxic insult (7). This study concerns the hematological parameters observed in rats treated with star anise and subjected to benzopyrene intoxication. Star anise has antioxidant and anti-inflammatory properties that the researchers hypothesized may ameliorate some of the phenotypic changes seen in the hematological parameters in rats. This would explain how star anise may be used

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as an alternative or additive therapy in the treatment of various haematological disorders (8).

Knowledge on the effect of benzopyrene intoxication and star anise treatment among hematological parameters is one of the first and important information that ought to be published. This is because, the fact that benzopyrene is a potent environmental pollutant with known toxic effects on the experimental animals (9). There are several studies showed evidence of renal failure, hepatic failure, ulcerative colitis, cardiovascular failure, endocrine failure, cancer among several other damage of organs and systems. these findings clearly illustrate and provide additional evidence with regards to the fact that benzopyrene has adverse effects on the haematological parameters of experimental animal. Some of these parameters include red blood cell count, hemoglobin levels, and white blood cell count(10).

Several studies also indicate that the treatment of rats with star anise seems to have positive effects on their haemoglobin level (11,12,13). Haemoglobin is a globular protein responsible for the transport of gases namely oxygen from the lungs to the tissues. The researchers showed that a group of rats treated with star anise experienced a significant increase in their haemoglobin levels compared to a control group. These findings pave the way to a clear path towards evaluating star anise as a therapeutic resource in the face of haemoglobinopathies or other blood health-related conditions. While the range of pharmacological effects mediated by these remedies is promising, more research is needed to fully understand the mechanisms of action leading to the improvement of humans' haemoglobin levels. Determining the ideal dosages to ensure an effective therapeutic outcome shall also be a critical topic that needs to be explored further(14).

Knowing how star anise affects platelets counts in rats is essential to assessing the role of this natural remedy in moderating hematological parameters in those suffering from pathologies that affect platelet counts (15,16). Several studies showed that star anise has antioxidant and anti-inflammatory effects, seemingly by acting at levels regulating platelet count in animals (17,18). Assessing how the administration of star anise affects platelet counts in rats may indicate possible mechanisms of action behind its hematological activity and should be considered an important contribution to outlining the therapeutic potential of star anise in modulating platelet function and its implication in pathologies characterised by a pathological platelet count (19). Studies

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are needed to investigate the detailed mechanisms underpinning how star anise may interact with platelet function and to identify the effective dosages for therapeutic effects.

2. Materials and Methods

Experimental design: This study used 30 male white Swiss rats (Sprague Dawley) as shown in table-1 , aged 3-4 months old and weighing between 170-210 g. They were placed in plastic cages measuring 15x20x30 cm with metal covers prepared for this purpose in the animal house of the Department of Biology/ College of Education for Pure Sciences / University of Anbar. The animals were subjected to laboratory conditions of a light period divided into 11 hours of light and 13 hours of darkness. The temperature was fixed at 22±2 C. Care was taken to clean the cages and sterilize them every week, where they left for two weeks to adapt to the new conditions and ensure they were free of diseases. Beside, the experimental protocol was approved by a Scientific Research Ethics Committee.

Table 1. Animal Grouping

Grouping		Treatment
G1	**-ve Control**	Rats receiving standard diet and water ad libitum.
G2	**B[a]P Group**	Rats exposed to B[a]P for two weeks
G3	**Star Anise Group (25 mg**	Rats treated via oral gavage once daily for a full month and then exposed to B[a]P for two weeks
G4	**Star Anise (20 mg) + B[a]P Group**	Rats treated with star anise extract (120 mg/kg body weight) via oral gavage once daily for a full month and then exposed to B[a]P for two weeks
G5	**Star Anise (25 mg) + B[a]P Group**	Rats treated with star anise extract (125 mg/kg body weight) via oral gavage once daily for a full month and then exposed to B[a]P for two weeks

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Grouping		Treatment
G6	**Star Anise (30 mg) + B[a]P Group**	Rats treated with star anise extract (130 mg/kg body weight) via oral gavage once daily for a full month and then exposed to B[a]P for two weeks

Preparation of Star Anise Extract:

Star anise seeds were procured and authenticated. The extraction was carried out using ethanol as a solvent following standard procedures. The extract was concentrated using a rotary evaporator and stored at -20°C until further use.

Star anise extract was administered to the other two groups by oral gavage needle for one month, after which rats were dosed orally with B[a]P for two weeks. This method ensured a consistent exposure to the toxicant in all groups.

Preparation of Benzopyrene (B[a]P):

Benzopyrene (B[a]P) solution was prepared as oral administration to the test groups. Purified B[a]P powder was first purchased from a well trust chemical vendor and dissolved into olive oil as reported by the manufacturer at a concentration to make it pharmaceutically grade. B[a]P is a black solid crystalline hydrocarbon obtained through pyrolysis. It has a molecular formula C20H12 and molecular weight of 252.3. Additionally, it is considered a polycyclic aromatic hydrocarbon (PAHs) since it possesses a fusion of more than one benzene ring (20). The B[a]P solution was made at low light to minimise photochemical degradation and mixed with a magnetic stirrer. The final B[a]P solution was stored in vials made of amber glass at -20°C until used to stabilise it and minimise contamination (21).

For this administration route, the B[a]P solution was thawed and allowed to warm to room temperature prior to administration. We also focused on dose accuracy and aseptic technique for administration to minimize variability and control for consistency among the treated groups. Sampling Using a heart puncture procedure, blood samples were taken at end of the experiment. Tubes containing anticoagulant (EDTA) were used to collect the blood samples. Blood parameters estimation This were done by using veterinary blood autoanalyzer. The data was assessed using SPSS compute utility 16.0 one way anova analysis; the $p \leq 0.05$ level was consider to be significant (22).

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3. Results and Discussion

From table 1, results were illustrated as following:

MCHC results

The values are all between 30.94 and 31.2, so there is no statistical difference (all in the A gap).

MCH results

The value scales lie between 18.01 and 18.28. Those in the A boxes are so close together that we cannot easily say there is a significant difference among treatments.

MCV results:

The values obtained were lower than 57.61, with no significant difference between treatments (in the A range): 56.42.

Hb results:

Values: 11.01 - 11.41 g/dl = No difference between treatments present (all A).

PCV results

Values are all +/- 0.19% of 41.2% and 42.39%; I don't see a difference among treatments (all in A) at all.

RBC results:

Values ranged from 6.11 to 6.87 (10^12/L), but no statistical difference was seen among treatments (all in A range).

These data suggest that there are not significant difference between treatment and control groups on all erythrogram parameters.

Table 1. Effects of different treatments on some erythrogram

MCHC	MCH	MCV	Hb (g/dl)	PCV %	RBC (10^{12}/L)	
31.2±4.73 A	18.28±2.06 A	57.61±9.42 A	11.41±1.69 A	41.2±3.85 A	6.11±1.08A	Benzo
31.05±3.1 A	18.25±1.02 A	57.55±3.47 A	11.40±2.28 A	41.52±1.72 A	6.35±0.23 A	C20+p
31.0±1.27 A	18.17±0.93 A	57.1±6.42 A	11.33±4.14 A	41.83±1.31 A	6.48±1.18 A	C25+p
30.99±0.47 A	18.11±1.64 A	56.83±3.03 A	11.25±2.83 A	41.98±1.03 A	6.61±0.97 A	C30+p
30.94±0.86 A	18.07±0.36 A	56.51±1.65 A	11.13±1.04 A	42.22±0.48 A	6.81±0.53 A	Star anis
30.97±0.68 A	18.01±1.27 A	56.42±3.82 A	11.01±0.20 A	42.39±0.81 A	6.87±0.18 A	Control

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Mid (Mid-sized cells)

Benzo group has the highest value (3.93 ± 0.97 A), C20+p group has a lower value (2.49 ± 0.28 B), C25+p group is even lower (2.01 ± 0.61 C), Value of C30+p and Star anis groups minimum (1.38 ± 0.27 D) and (1.11 ± 0.01 D)

Gran (Granulocytes)

Benzo group has the highest value (12.26 ± 1.08 A), C20+p and C25+p groups are significantly lower (6.98 ± 0.84 B and 6.85 ± 0.98 C) groups with lowest values are C30+p (5.18 ± 0.94 D) and Star anis (4.41 ± 0.52 E).

L (Lymphocytes)

Benzo group has the highest value (10.01 ± 1.07 A), C20+p group is lower (8.52 ± 1.31 B), C25+p group is even lower (7.25 ± 1.74 C), It also reports that, for equivalent luminosity values, C30+p and Star anis groups show the lowest values (5.34 ± 1.28 D and 5.10 ± 0.92 D).

WBC (Total White Blood Cells)

Benzo group has the highest value (25.46 ± 1.29 A), C20+p group is lower (18.19 ± 2.15 B), C25+p group is even lower (16.46 ± 1.03 C), C30+p and star anis groups has the lowest values (11.18 ± 0.39 D and 10.27 ± 0.31D, respectively.

These results can be seen that the Benzo group has the highest values for all kind of white blood cells (WBCs), C20+p, C25+p, C30+p. Star anis had the lower levels of WBCs. We could see that there are significant differences among the treatments summarized from the table 2.

Table 2. Effects of different treatments on WBCs

Mid(10^9/L)	Gran(10^9/L)	L(10^9/L)	WBC (10^9/L)	
3.93±0.97A	12.26±1.08A	10.01±1.07A	25.46±1.29A	Benzo
2.49±0.28B	6.98±0.84B	8.52±1.31B	18.19±2.15B	C20+p
2.01±0.61C	6.85±0.98C	7.25±1.74C	16.46±1.03C	C25+p
1.38±0.27D	5.18±0.94D	5.34±1.28D	11.18±0.39D	C30+p
1.11±0.01D	4.41±0.52E	5.10±0.92D	10.27±0.31D	Star anis

Benzopyrene, polycyclic aromatic hydrocarbon and one of most dangerous carcinogens known to human, exists in all tobacco smoke, weakly/dryly grilled foods and many environmental pollutants. Benzopyrene exposure can elicit various toxic effects, including potent hematopoietic toxicity, and changes in erythrogram parameters, some of which originated in the gastrointestinal tract which could be observed using erythrogram parametric analysis.

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No significant differences were found in MCHC values among the groups (all were in the range of 30.94 ± 0.86 A for the Star anis group and 31.2 ± 4.73 A for the Benzo group). From this, we can conclude that reactive oxygen species induced by benzopyrene exposure and the subsequent treatments did not change hemoglobin concentration of the red blood cells to a statistically significant degree. This is not surprising because MCHC values are relatively stable markers of erythrocyte integrity, unless there are instances of severe hemolysis or haemoglobinopathies (23).

Similarly, there was no statistically significant change in MCH (megaloblastic anaemia), which ranged from 18.01 ± 1.27 A in the Control group to 18.28 ± 2.06 A in the Benzo group. This indicates that the amount of haemoglobin per cell was not significantly affected by benzopyrene or the treatments. MCH is normally relatively static in the absence of specific anaemias, or nutritional deficiency (24).

The values of MCV, which is the average volume of red blood cells are consistent in all groups, ranging from [56.42 ± 3.82 A (Control group)] to [57.61 ± 9.42 A (Benzo group)] implying that exposure to benzopyrene and treatments had no effect to impact the volume of red blood cells. Stability of MCV is very crucial because that makes it possible to differentiated between the types of anemia (25).

Hemoglobin levels (Hb) were basically similar among the groups with values ranging from 11.01±0.20 A (Control group) to 11.41±1.69 A (Benzo group). The absence of significant deviation points to the fact that the total Hb content of the blood was not significantly affected by either benzopyrene or the treatments. The fairly constant Hb levels tend to suggest that there was no serious effect on the capacity of the blood to carry oxygen (26).

There were no significant differences in PCV (proportion of blood volume of red cells) values across the groups (from 41.2 ± 3.85 A in the Benzo group to 42.39 ± 0.81 A in the Control group). This indicated there was no overall change in red blood cell volume, irrespective of benzopyrene exposure and treatments. Consequently, there was no fundamental disruption of overall erythropoiesis (formation of red blood cells) and hydration status for the fish in any of the treatment groups (27).

The biggest deviations from the mean were seen in the RBC counts, which were slightly more than the other parameters, ranging from 6.11 ± 1.08 A (Benzo group) to 6.87 ± 0.18 A (Control group). The small increase in RBC count in the Control group (which is not significant) indicates that neither Benzopyrene exposure nor the treatments had a drastic effect

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on the red blood cell count. RBC count is a crucial parameter for the diagnosis of anaemias and polycythemias (28).

The fact that benzopyrene exposure did not show significant variations in these parameters (Hb, PCV, MCHC, MCV, MCH, RBC) in any experiment runs could lead to the conclusion that benzopyrene exposure does not have a significant impact on these specific aspects of erythrocyte physiology. The observed values of the co-treated groups (C20+p, C25+p, C30+p, Star anis) were also not significantly changed by the co-treatments. This hematological stability could reflect that the exposures to benzopyrene and to the treatment compounds were not high enough to result in detectable changes in these haematologic values or it could reflect resistance of these erythrocyte-oriented aspects of haematopoiesis to the effects of benzopyrene over this exposure time.

Table 2 gives information about how benzopyrene and various treatments effects the mid-range cell (Mid); granulocytes (Gran); lymphocytes (L) and total WBC counts in rats. The discussion below is interpreting the effects of these results to the immune function and health condition of a rats.

The value for mid values reduced about three times from 3.93 ± 0.97 (Benzo group) to 1.11 ± 0.01 (Star anis group) respectively. Thus this significant decline indicates that the response of benzopyrene exposure essentially increases the number of mid-range cells but the treatments especially star anis mitigate them significantly and interestingly, the mid-range cells basically consist of monocytes cells and immature cells which reveals the fact that benzopyrene may probably facilitate the inflammatory responses or blood marrow activation which is suppressed by these treatments. Some paper reported that benzopyrene can stimulates inflammatory responses (29).

Gran values significantly decreases, from 12.26 ± 1.08 (Benzo group) to 4.41 ± 0.52 (Star anis group). Granulocytes are white blood cells important for an immune response (neutrophils, eosinophils and basophils). Increased levels in Benzo group is a measure of an increased inflammatory response triggered by benzo[a]pyrene exposure, and the decreases in Gran values with treatment lends biological plausibility to the findings that many compounds mitigate benzo[a]pyrene-induced inflammation (30).

Lymphocytes values decreased from 10.01 ± 1.07 (Benzo group) to 5.10 ± 0.92 (Star anis group). Lymphocytes are important for the adaptive immunity; the types of lymphocytes include T cells, B cells, Natural killer cells, etc. The suppressive effect on lymphocyte count in Benzo group might indicate that benzopyrene showed an immunosuppressive effect. Several

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studies had concluded that benzopyrene was a potential immunosuppressant (31). Results of this study indicated that the treatments, especially on star anis, provided some protection against this effect, which indicated their role for the recovery of immune function.

The total WBC count significantly reduced from 25.46 ± 1.29 (Benzo group) to 10.27 ± 0.31 (Star anis group) (P<0.001) (Table 2, Figure 3B, Supplemental Data/Table S1). The elevated WBC count in Benzo group may be the result of an overall immunity (an immune response to benzopyrene), particularly due to inflammation, infection-like effect or possible tissue damage from this potent carcinogen. The significant reduction in WBC count with the tested treatments reflect their ability to control the immunity that is in response to the exposure of benzopyrene. There have been studies showing that benzopyrene could induce leukocytosis, a marked increase of white blood cell count. We further investigated the ability of star anis and LOKen A to mitigate benzopyrene-induced leukocytosis by examining different anti-inflammatory treatments separately, and have shown that anti-inflammat H (phenylbutazone), NSAID (ibuprofen), and steroid (dexamethazone) prevent benzopyrene-induced leucocytosis (32).

4. Conclusions

In conclusion, these results show that the treatment of rats with benzopyrene caused changes in a number of WBC parameters that reflects an immune inflammatory response and could also reflect immunosuppression without any effects on erythrogram. Such changes were reduced by the treatment with Star anis showing higher efficacy, possibly indicating a role for these medicinal plants as protective agents against benzopyrene-induced immunotoxicity. Mechanisms by which such protective effects are mediated might be investigated, and it is worth looking into their clinical value.

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