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Ameliorative effect of Chamomile flowers extract against thinner inhalation-induced hematotoxicity in rats

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Abstract: Volatile organic compounds in thinner are considered harmful to the environment and occupational workers. One of the adverse health effects of thinner exposure is hematotoxicity. This study aimed to study the hematotoxic effects of chronic thinner inhalation and the possible protection by chamomile extract administration. Adult male Wistar rats were exposed to thinner fumes for 8 weeks (4 hours/day, 6 days/week), while chamomile flower extract (400mg/kg b.w) was given orally during thinner exposure for the same period. The study showed significant decreases in RBCs count, Hb content, HCT%, MCV, MCH, and PLTs count in thinner exposed group comparing with control group. Similar decreases were observed in total and differential WBCs count, except for neutrophils that were increased significantly with reference to control group. Administration of chamomile with thinner exposure showed a reverse behavior for all tested parameters on comparing with thinner exposed group. Therefore, consumption of chamomile extract had an ameliorative effect against thinner inhalation induced hematotoxicity in rats.

keywords: Thinner; hematotoxicity; Chamomile

1.Introduction

Thinner is one of the commonly used industrial solvents which introduced in furniture, paint, automobile manufacture and repairs occupations. Those living around these workplace environments are at the risk of exposure to its constituents ubiquitously released into the environment **[1]**. Several studies have investigated that exposure to thinner is associated with several adverse health effects such as neurotoxicity, hepatotoxicity, renal toxicity, as well as respiratory diseases **[2-4]**. Thinner contain many VOCs like toluene, xylene, etc. these VOCs and other metabolites are known to be hematotoxic, they are known to have a deleterious effect on bone marrow. These effects may results in decreased production of red blood cells, white blood cells, and platelets counts **[5]**. Occupational exposure to constituents of paint thinners such as toluene has a significant decrease in circulating erythrocytes, haemoglobin, platelets, total white blood cells, and absolute numbers of lymphocytes and neutrophils **[6]**.

Back to nature is a concept in many recent researches. Traditional medicinal plants are often cheaper, locally available, and easily consumable **[7]**. Usage of these plants instead of synthetic drugs often mediate beneficial responses due to their active chemical constituents **[8]**. Chamomile (Matricaria chamomilla) is one of the most common plants used in medicinal field **[9-11]**. Pharmacological investigations referred that chamomile has several biological activities **[12]**. Chamomile used in modern medicine primarily for their spasmolytic, antiphlogistic, and antibacterial properties. It is also used as a multipurpose digestive to treat gastrointestinal disturbances including flatulence, indigestion, diarrhea, anorexia, motion sickness, nausea, and vomiting **[7]**. **[13]** reported that terpenoids, including bisabolol and its oxides A&B, bisabolone oxide A, chamazulene and β-farnesense are the most important compounds group in chamomile essential oil. On the other hand, chamomile extracts are dominated by phenolic compounds such as phenolic acids,

flavonoids and coumarins **[10]**. **[14]** found that the analysis of chamomile oil, obtained by hydro-distillation, using Gas chromatography showed that the major components were linoleic acid (54.8%), oleic acid (23.5%), palmitic acid (10.7%) and linolenic acid (6.2%).Also, **[15]** found the main components identified in the chamomile essential oil using GC-MS analysis ; camazulene (19.9%), α-bisabollo (20.9%), A and B bisabolol oxides (21.6%, and 1.2% respectively).The objective of this study was to investigate the effectiveness of chamomile flower extract administration against hematotoxicity effects of chronic thinner inhalation in rats .

2. Materials and methods

2.1. Animals and studied groups

Thirty adult male Wister albino rats, weighing 160 -170 g, obtained from the Egyptian Institute for Serological and Vaccine Production, Helwan, Egypt were used in this study. Animals were housed in a well-ventilated animal house and kept under standard environmental conditions (23±2 °C room temperature, 40±5% humidity, and 12 hr light/dark cycle) in stainless steel cages provided with food and water *ad libtium*. Animals were acclimated to the new environment for one week, then divided randomly into 5 groups (6 rats/ each):Group I served as control (CN); Group II, served as vehicle (VE) and received distilled water (1ml/kg b.w) orally by gavage; Group III received chamomile (CM) extract (400mg/kg b.w) dissolved in 1ml distilled water and supplemented through oral gavage, according to **[16]**; Group IV was exposed to thinner (TH) fumes (4 hr daily, 6 days/week), repeatedly for 8 weeks and Group V was exposed to thinner fumes and received chamomile extract (TH+CM) as described above. All procedures were approved by the Ethics Committee for Animal Research of Mansoura University, Egypt (Ph-Z-2020-1).

2.2. Thinner exposure

Animals were exposed to thinner fumes through a whole-body inhalation chamber as previously described by **[17]**. Used thinner (Dababa trademark) was put at the bottom of the exposure chamber in two open calibrated beakers, each containing 500 ml of thinner at ambient temperature. The volume of the liquid thinner was recorded daily before and after the period of exposure to calculate the released volume that was inhaled by rats. The average evaporated volume during the thinner exposure period in each day was about 18 ml from two beakers. Non-exposed animals (Groups I, II, III) were maintained in the inhalation chamber for the same period and conditions, but without thinner exposure. Chemical analysis of used thinner showed more than 50 volatile compounds, according to our previous study **[18]**, where the most representative compounds were toluene (39.5%), xylene (34.9 %), ethanol-2-butoxy (5.27%), methyl acetate (3.68%), sec-butyl acetate (2.60%), benzene-1-ethyl-3-methyl (2.5%), benzene-1-ethyl-2-methyl (2.17%), benzene-1-2-3-4-tetramethyl (1.99%), propyl benzene (1.10%) and benzene-1-2-4-trimethyl (1.0%). However, the remaining compounds were less than 1% each and were not represented.

2.3. Plant extraction and GC-Ms analysis

Dry chamomile plant was bought from local market at Mansoura City. An expert in the botany department of the Faculty of Science, Mansoura University, Egypt, identified the dried chamomile plant used in this experiment as *Matricaria chamomilla*. Chamomile flowers with the smallest amount of stalk were weighed and ground into a fine powder in a mortar. Distilled water was added to a flask to create a 5% suspension (w/v). After that, the flask was placed atop an electric shaker (57 xg) and kept at 37°C for 4 hours. The liquid was stirred, cooled to room temperature, then filtered using a series of Whatman filter papers to produce an aqueous infusion. The filtered aqueous extract was frozen at -20°C until used. Chamomile extract was administrated to rat groups according to **[16].**

Essential oil of chamomile flower extract were performed using gas chromatography mass spectrometer "GC-MS" (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to250 °C hold for 2 min. increased to the final temperature 300°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were

injected automatically using Autosampler AS1300 coupled with GC in the split mode. Ionization energy EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST 14 mass spectral database **[19]**.

2.4. Animals investigation

At the end of the experimental period (8 weeks) and after 24 hr from the last thinner exposure, all rats were sacrificed under anesthesia with intraperitoneal injection by a combination of ketamine (80 ml/kg b.w) and xylazine (8 ml/kg b.w) **[20]**. Blood samples were collected from each rat on EDTA to determine complete blood count (CBC), total and differential leucocytes counts by using the fully automatic hematological analyzer (Sysmax XE-2100, Japan) according to **[21]**.

2.5. Statistical analysis

The GraphPad Prism software program (v 5.04 GraphPad Software Inc., La Jolla, CA) was used to analyze the obtained data using one-way ANOVA followed by Tukey's test, where results were expressed as the mean ± SD, and statistically significant data were considered at *p*-values < 0.05.

3. Results

3.1. Chamomile GC-MS analysis

Table (1) and Fig. (1) showed that GC-MS analysis of the chamomile extract separated more than 50 volatile compounds from different classes. The highly found compounds were arranged from the highest percentage to the lowest as follow: α-Bisabolol oxide A (65.54%), Linolenic acid (28.05 %), (E)-Tonghaosu (2.76%), E-Tibetin spiroether (1.80 %), α-Bisabolol oxide B (0.94 %), (Z)-Tonghaosu (0.52 %), Linolenic acid, methyl ester (0.33 %).

3.2. Animal investigations

3.2.1. Complete blood count (CBC)

As shown in Figs. 2 & 3, significant decreases in RBCs count, Hb content, HCT%, MCV, MCH, and PLTs count were observed in thinner exposed group compared to control group. Similar decreases were noticed in total and differential WBCs count, except for neutrophils that increased significantly compared to control group. Administration of chamomile with thinner exposure showed a reverse behavior, comparing with thinner exposed group. RBCs count, Hb content, HCT%, MCV, MCH, and PLTs count were increased significantly compared to thinner exposed group. Additionally, total and differential count of WBCs, except for neutrophils were significantly increased with chamomile administration during exposure compared to thinner exposed group. On the other hand, there were no significant changes in all parameters in the normal animal group received chamomile or vehicle (water) comparing with the control group.

Table 1: Natural components and their relative abundances in chamomile through GC-MS analysis

Relative abundance (%)	Retention time	Natural components	Code no.
0.94	17.73	α-Bisabolol oxide B	1
65.54	20.41	α-Bisabolol oxide A	2
2.76	22.99	(E)-Tonghaosu	3
0.52	23.35	(Z)-Tonghaosu	4
1.80	23.49	E-Tibetin spiroether	5
0.33	26.62	Linolenic acid, methyl ester	6
28.05	29.54	Linolenic acid	7

Retention time: is the amount of time taken for a solute to pass through chromatography column.

Relative abundance: the percent area under the peak which measures the concentration of the compound.

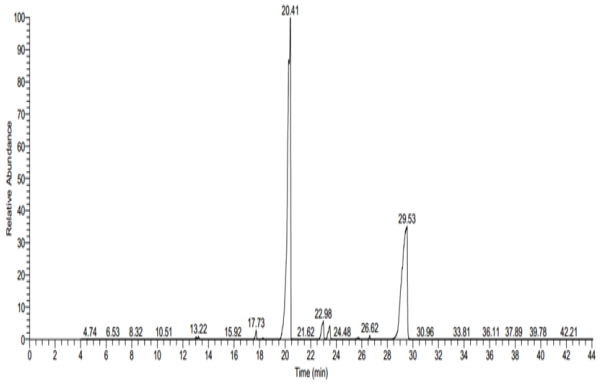

Fig. 1: Chromatogram of chamomile components by GC-MS analysis

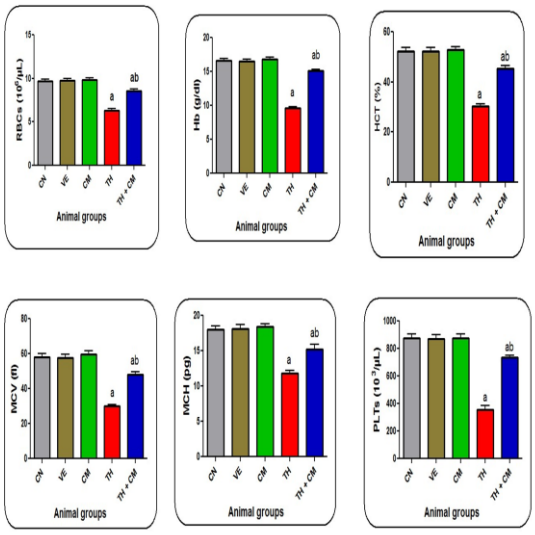

Fig.2. Changes in RBCs indices and platelets count in control and different rat groups. a: significant difference from control group ($p< 0.05$). b: significant difference from thinner group ($p<0.05$).

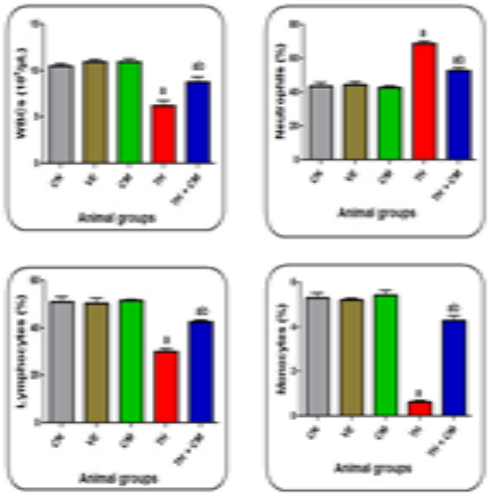

Fig.3. Changes in total and differential count of WBCs in control and different rat groups. a: significant difference from control group ($p< 0.05$). b: significant difference from thinner group ($p<0.05$).

4. Discussion

Hematological indices have often been used to assess the state of health of a given organism exposed to toxicants in a particular environment. One of the adverse health effects of thinner exposure is hematotoxicity, which is mainly related to different aromatic constituents of thinner. Toluene is one of these constituents that can rapidly be distributed throughout the body, mostly to the adipose tissue, bone marrow, where it induces physiological and immunological changes **[5]**. **Abdrabouh *et al*. [22]** attributed the disturbance in blood cells to the released reactive oxygen species (ROS) results from exposure to volatile organic compounds. The authors added that ROS can directly damage RBCs membranes thus affecting the RBCs flow and other blood indices. On the other hand, indirect effect may result from affecting bone marrow that is responsible for hematopoiesis. This agreed with the study of, where marked decreases in RBCs count, Hb, and platelets in mice exposed to thinner fumes were observed. Thinner hematotoxicity also extended to leucocytes which reduced significantly with thinner exposure. **[1]** related this decrease to the affected bone marrow.

The usage of chamomile as a natural plant for possible amelioration in blood indices after exposure to thinner fumes has resulted in a promising results represented by significant amelioration of the detected blood indices. The present study showed GC-Mass analysis of chamomile with several essential oils that mainly represented by α-Bisabolol oxide A and linolenic acid. This was agreed with several previous studies which confirmed that essential oils in chamomile have antioxidant potential and immune boosting due to its free radical scavenging activity **[7,23,24]**. In turn, this may reduce the hematotoxicity through reducing myelotoxicity as observed by **[9]**. Another studies supported these results, referring to the high ability of fatty acids such as linolenic acid in chamomile to actively promote the proliferation in an in vitro hemopoietic stem cell in anemic mice. Authors also found that linolenic acid had a marked stimulatory effect on bone marrow cells leading to hematopoietic recovery **[25, 26]**.

5. Conclusion

In conclusion, chamomile flower extract is rich in several essential oils, especially linolenic acid. Administration of chamomile water extract during exposure to thinner fumes has participated in the amelioration of deteriorated blood parameters when compared to the exposed group.

6. References

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