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POTENTIAL IMPACTS OF SAGE (*SALVIA OFFICINALIS* L.) LEAVES EXTRACT AND *XANTHOPHYLLOMYCES DENDRORHOUS* CULTURAL FILTRATE ON DIMETHYLFORMAMIDE INDUCED PHYSIOLOGICAL RESPONSES AND HEMATOTOXICITY IN MICE

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ABSTRACT

The purpose of this study was to assess the potential effects of *Salvia officinalis* ethanolic leaves extract (SEE) and *Xanthophyllomyces dendrorhous* culture filtrate (XDCF) on physiological and hematological responses in mice injected interperitoneally with dimethyl formamide (DMF). Twelve groups comprised equally of 36 male and female Swiss albino mice were divided into 6 groups of males and 6 groups of females were represented (3 animals each). The experimental administrations were the same for the male and female groups. The first group was the control group. Every day for 21 days, the second, third, and fourth groups received 378 mg of DMF intraperitoneally, 100 mg of SEE orally, and 3300 µl of XDCF intraperitoneally / kg body weight, respectively. The fifth group received 100 mg of SEE / kg body weight orally every day for a further 21 days after receiving 378 mg of DMF / kg body weight intraperitoneally every day for 21 days. Following a 21-day intraperitoneally exposure to 378 mg of DMF / kg body weight per day the sixth group was given daily XDCF intraperitoneally by 3300 µl / kg body weight. In response to DMF administration, the percentages of the liver, kidney, and heart significantly increased while the percentages of the spleen and lung significantly decreased in both males and females mice compared to the control group. RBCs, Hb%, and PCV% were hematological indicators that significantly

reduced due to DMF administration. SEE and XDCF alleviated the adverse effects of DMF on growth performance indicators, various organs, and hematological parameters. These results demonstrate the potential of SEE and XDCF as anti-toxin agents with a broad range of beneficial health impacts.

Key words: dimethylformamide, hematotoxicity, mice, physiology, sage.

INTRODUCTION

Medicinal herbs have been known as rich sources of pharmacologically active compounds. Herbs are present in 40% of prescription medications today **(Newman and Cragg, 2007)**. *Salvia officinalis* L. belongs to the *Lamiaceae* family, *Nepetoideae* subfamily, *Mentheae* tribe, and *Salvia* genus **(Dinç *et al*., 2009)**. Salvia is the biggest *Lamiaceae* genus, with over 1000 species **(Walker *et al*., 2007)**, and can be found throughout Europe, Southeast Asia, Central and South America and Africa **(Ulubelen, 2000)**. Due to its flavor and digestive properties, *S. officinalis* has been utilized in food preparation for a very long time. Sage extracts are beneficial as natural preservatives in food, cosmetics, and medicinal items due to their antioxidant properties **(Bouajaj *et al*., 2013 and Prakash *et al*., 2015)**. Many biological effects, including anti-inflammatory, neuroprotective, cytotoxic, anti-bacterial, anti-fungal, and antiviral, have been associated to salvia species **(Asadi *et al*., 2010; Orhan *et al*., 2013** and **Ben Farhat *et al*., 2014)**. The main phytochemicals found in *S. officinalis* flowers, leaves, and stems are well known. Alkaloids, carbohydrates, fatty acids, glycosidic derivatives, phenolic compounds, poly acetylenes, steroids, terpenes/terpenoids, and waxes are among the diverse constituents found in *S. officinalis* **(Velickovic *et al*., 2003; Badiee *et al*., 2012 and Russo *et al*., 2013)**.

The basidiomycetous yeast *Xanthophyllomyces dendrorhous* (sexual stage *Phaffia rhodozyma*) forms pink to red colonies and possesses several special characteristics not found in other yeast species. Its primary carotenoid pigment, astaxanthin, is a pigment that is not detected by observed amount in other yeasts. Due to its impressive antioxidant, neuroprotective, anti-inflammatory, and anticancer qualities, astaxanthin is the second-most significant carotenoid on the market **(Schmidt *et al*., 2011)**. The polyene chain and many double bonds found in astaxanthin quench singlet oxygen and radicals to terminate the process. The chemical and physical interactions of antioxidants with cell membranes have been connected to their antioxidant effects. Astaxanthin's polyene chain scavenges free radicals in the cell membrane **(Ambati *et al*., 2014)**. Therefore, the current study was conducted to evaluate the prospective impacts of *Salvia officinalis* ethanolic leave extract (SEE) and *Xanthophyllomyces dendrorhous* cultural

filtrate (XDCF) on growth performance indicators, physiological and hematological responses in mice interperitoneally exposed to dimethyl formamide.

MATERIALS AND METHODS

Sage (*Salvia officinalis* L.)

Fresh sage plants were purchased from the commercial market in Minya Governorate. A botanist from the horticulture department (ornamental plants branch) authenticated plants using their botanical characteristics and identified them as *Salvia officinalis*. Manual cleaning with clean water and removal of foreign substances and debris were applied to sage plants. At the department of agricultural chemistry, leaves were manually collected from plants and air dried for roughly 3 weeks until they reached a constant weight. The dried leaves were crushed into a fine powder in a Braun mill (Germany), sieved (40 mesh), and then kept in dark plastic bags at 25°C for future use.

Xanthophyllomyces dendrorhous isolate and cultural filtrate.

Xanthophyllomyces dendrorhous (formerly *Phaffia rhodozyma*) strain: NRRL Y-17269 [VKM Y-2268] from the American Type Culture Collection (ATCC) Manassas, VA 20108 USA. Yeast was provided by the Agricultural Microbiology department, Faculty of Agriculture, Minia University. The *Xanthophyllomyces dendrorhous* isolate was grown in YM broth media for 10 days in 250 ml flasks containing 50 ml of the medium in an incubator shaker at 22°C and 200 rpm (**Kalyanee *et al.*, 2007**). The culture media included 1000 ml of distilled water, 3g of yeast extract, 3g of malt extract, 5g of peptone, 10g of dextrose, and 3g of peptone. Following 10 days of shaking, the culture broth was filtered to separate the cultural filtrate using Millipore filter paper (0.22 µm).

N, N-dimethylformamide reagent [$(CH_3)_2NC(O)H$].

The N, N -dimethylformamide reagent (CAS No. 68-12-2), Purity ≥ 99.8, was purchased from Oxford Lab Fine Chem in Maharashtra, India. According to **Hellwig *et al.*, (1991)**, the reagent was administered as a practical dose for the experiments at 378 mg / kg body weight.

Preparation of Sage (*Salvia officinalis*) leaves extracts.

Salvia officinalis dry leaves weighing 15g were extracted by 250 ml methanol using a Soxhlet extractor at 65°C for 6 hours to prepare a sage methanolic extract **(SME) (Ajeel *et al.*, 2021)**. To prepare sage ethanolic extract **(SEE)** and sage hexane extract **(SHE)**, 20 g of the dry leaves of *Salvia officinalis* were extracted by 250 ml ethanol 99.8%, and 250 ml hexane 99.5% in a Soxhlet apparatus for 6 hours at 78°C and 69°C respectively. The all extracts were then filtered using Whitman filter paper and dried until a consistent weight after the solvents were evaporated in a rotary evaporator **(Altalhi *et al.*, 2018).**

Folin-Ciocalteu method for total phenolic content (TPC).

The Folin-Ciocalteu reaction is a spectrophotometric technique based on the ability of polyphenols to generate colored reactions with the Folin-Ciocalteu reagent, and it is an

antioxidant assay that assesses the reductive capacity of an antioxidant **(Chun *et al.*, 2003)**. The absorbance corresponds with the sample's total phenolic component concentration. The process measures were utilized to guarantee optimal outcomes for our study: 25 µl of each sage leaves extract (SME, SEE and SHE) was combined with 25 µl of Folin-Ciocalteu reagent. After being held at 25°C for 5-8 minutes, the volume of the solution was increased to 200 µl with distilled water, and 25 µl of 20% sodium carbonate solution was added. A spectrophotometer was used to measure the absorbance at a wavelength of 750 nm after 60 minutes. A gallic acid standard curve was used to determine the total phenolic content. The results are expressed as mg gallic acid equivalents per gramme of extract (mg GAE/g extract) **(Pior *et al.*, 2005)**.

Total flavonoids content (TFC).

A colorimetric reaction was used to determine the total flavonoids content using the aluminum chloride method. The following elements were added to the original technique to fit our research: 75 µl of ethanol 99.8% were combined with 25 µl of each sage leaves extract (SME, SEE and SHE). After that, 10 µl of 1M potassium acetate were added. 10 µl of 10% $AlCl_3$ were added after five minutes. As soon as possible, 130 µl of distilled water was added to the mixture and properly stirred. After 30 minutes of incubation in the dark, the absorbance was measured at 510 nm against the blank. A quercetin standard curve was used to measure the total flavonoid content. The results are expressed as mg of quercetin equivalents per gramme of extract (mg QCE/g extract) **(Djeridane *et al.*, 2006)**.

Total phenolic acids content (TPAC).

Arnov's reagent is used in the spectrophotometric Arnov method, which **Gwalik-Dziki *et al.*, (2009)** describes as a method for determining the amount of total phenolic acid in a sample. Briefly, 25 µl of each sage leaves extract (SME, SEE and SHE) were combined with 150 µl of distilled water, 25 µl of 0.5N HCl, 25µl of Arnov's reagent (10g of sodium molybdate and 10g of sodium nitrate dissolved in 100 methanol), and 25µl of 1N NaOH. The absorbance values were measured at 490 nm. A caffeic acid standard curve was used to measure the total phenolic acids content. The results are expressed as mg of caffeic acid equivalents per gramme of extract (mg CAE/g extract).

Total carotenoid content (TCC).

The total carotenoid content of yeast was estimated according to **An *et al.*, (1996)** method. In brief, 1 ml of yeast culture was taken and resuspended in distilled water before being centrifuged to separate the cell pellet. Glass beads and 1ml of DMSO were added and then a vortex mixer was used to break up the cells. Then, vertexing was done after adding 1 ml at a time of acetone, petroleum ether, and 20% (w/v) NaCl solution to perform the carotenoid extraction. After the solvent layers had settled, a visual spectrophotometer was used to measure the carotenoid in the petroleum ether layers' absorbance at 474 nm (A474). The average total carotenoid of each sample was calculated after it was carried out in triplicate. The total

carotenoid content was calculated by using the following formula:

$$\text{Total carotenoid } (\mu g/g \text{ of yeast}) = \frac{\text{ml of petrol} \times A474 \times 100}{21 \times \text{yeast dry weight}}$$

Activity of scavenging hydrogen peroxide.

In phosphate buffer, a 40 mM H_2O_2 solution was prepared (pH 7.4). Each sage leaves extract (SME, SEE and SHE) and *Xanthophyllomyces dendrorhous* cultural filtrate was combined with 0.6 mL of 40 mM H_2O_2. After 10 minutes of incubation at 37°C, the absorbance of H_2O_2 at 230 nm was compared to the absorbance of a blank solution containing phosphate buffer but no H_2O_2. After that, the following formula was used to determine the percentage of H_2O_2 scavenging. **(Khan and Akhtar, 2003)**.

$$\% \text{ Scavenging of } H_2O_2 = \frac{Ac - As}{Ac} \times 100$$

Where:

Ac = absorbance of the control in the absence of the sage leaves extracts or *Xanthophyllomyces dendrorhous* cultural filtrate

As = absorbance in the presence of the sage leaves extracts or *Xanthophyllomyces dendrorhous* cultural filtrate

Anti-inflammatory activity (*In vitro*).

Using the use of a modified inhibition of albumin denaturation experiment, the anti-inflammatory potential was evaluated. **(Mizushima and Kobayashi, 1968)**. 50 mg of *Xanthophyllomyces dendrorhous* culture filtrate or each sage leaf extract (SME, SEE, and SHE) were dissolved in 1 ml of 2.5% dimethyl formamide (DMF) and diluted with 0.2M phosphate buffer to create the test solution (pH 7.4). In 0.2M phosphate buffer, 1 ml of the test solution was combined with 1 ml of 1 mM albumin. The mixture was then incubated in an incubator at 27°C for 15 minutes. To cause denaturation, the reaction mixture was heated in a water bath to 60°C for 10 minutes. Spectrophotometry was used to estimate the turbidity at 660 nm. The following formula was used to determine the percentage inhibition of denaturation:

$$\% \text{ inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where:

Ac = absorbance of the control in the absence of the sage leaves extracts or *Xanthophyllomyces dendrorhous* cultural filtrate

As = absorbance in the presence of the of the sage leaves extracts or *Xanthophyllomyces dendrorhous* cultural filtrate

Animals and experimental design

The Agricultural Chemistry Department, Biological Experimental Animal Lab, Minia University, Al-Minya, Egypt provided 36 Swiss albino mice, 18 males and 18 females, aged 8 weeks, with an average weight of 27 ± 2 g. The animals were housed in separate plastic cages in a climate-controlled condition with a constant temperature of (21–25 °C), a humidity level of (50–60 %), and a 12-hour photoperiod. A commercial balanced diet and tap water

ad libitum were supplied. After acclimatization for one week, animals were divided into twelve groups included 6 groups of males and 6 groups of females (3 animals each). To conducting the experiment 42 days were dedicated. For both the male and female groups, the experimental administrations were the same. The control group was the first group. The second, third, and fourth groups received 378 mg of DMF intraperitoneally, 100 mg of SEE orally, and 3300 µl of XDCF intraperitoneally / kg body weight every day for 21 days respectively. The fifth group received 378 mg of DMF / kg body weight intraperitoneally every day for 21 days followed by 100 mg of SEE / kg body weight orally every day for another 21 days. Following a 21-day intraperitoneally exposure to 378 mg of DMF / kg body weight every day the sixth group was given daily XDCF intraperitoneally by 3300 µl / kg body weight.

Physiological assessments

Indices of growth performance

The initial and final body weights were measured at the beginning and end of the 42-day experiment. The following formulas were used to calculate body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (FE): **(Suyitman *et al.*, 2020).**

Body weight gain (BWG) = final weight – initial weight

$$\text{Average daily gain (ADG)} = \frac{\text{Final weight} - \text{initial weight}}{\text{Number of experimental days}}$$

Average daily feed intake (ADFI) = feed provided - feed remaining

$$\text{Feed efficiency (FE)} = \frac{\text{Average daily gain}}{\text{Daily feed intake}} \times 100$$

Relative weights of various organs

The following equation was used to calculate the relative weights of the various organs (liver, kidney, spleen, heart, lung, and brain) at the end of the experiment:

$$\text{Organ relative weight (\%)} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Blood sampling and hematological investigations

After 42 days of the experiment, three mice from each group were weighed, killed, and allowed to bleed out fully. At 7:00 and 8:00 a.m., decapitation was utilized to collect blood in heparinized tubes while avoiding first drips. An animal hematology analyzer was utilized to assess red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), the standard deviation of red blood cell distribution width (RDW-SD), coefficient of variation of red blood cell distribution width (RDW-CV), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW), procalcitonin (PCT), platelet large cell ratio (P-LCR), white blood cells (WBCs) count, lymphocytes (%) and neutrophils (%)

Statistical analysis

The data were analyzed statistically using IBM SPSS Statistics version 25 software. Values are displayed as the mean ± SEM. One-way analysis of variance (ANOVA) and Duncan's multiple comparison tests were used, and the results of the comparison between the control and treatment groups were deemed statistically significant with a 95% confidence interval of $P \leq 0.05$.

Ethics committee approval

All procedures that were carried out in accordance with the guidelines for the care and use of laboratory animals were approved by the Ethics Committee for the care and use of animals, microorganisms, and living cell cultures in education and scientific research at the Faculty of Agriculture, Minia University, and were given the approval number: MU/FA/005/12/22.

RESULTS AND DISCUSSION

The phytochemical content of sage (*Salvia officinalis*) leaves extracts.

Many compounds that are responsible for the pharmacological activity of plants and are unequaled by those of synthetic compounds are known to be produced by plants. In a while, a large variety of phytochemicals that are found in one species are frequently only partially responsible for the activities. phenolic compounds are one of the most common groups of plant-based substances **(Graczyk *et al.*, 2022).** Many plant tissues contain phenolic substances called flavonoids. These are phytoconstituents having the molecular structure C_6-C_3-C_6, which consists of two aromatic rings joined by a three-carbon link **(Agati *et al.*, 2011)**. In plants, phenolic acids, which are found in both free and bound forms, are a prominent class of phenolic compounds. The subgroups of hydroxybenzoic acid and hydroxycinnamic acid can be used to categorize phenolic acids **(Zhang *et al.*, 2016)**.

Plant phenolic compounds represent specific antioxidant and anti-inflammatory properties of sage. Therefore, the first goal of this study was to assess the total phenolic content (TPC), total flavonoid content (TFC), and total phenolic acids content (TPAC) of various sage leaves extracts. These values are shown in **Figure 1** and are measured as mg gallic acid equivalent/g extract, mg quercin equivalent/g extract, and mg caffeic acid equivalent/g extract, respectively.

In the sage methanolic extract (SME), sage ethanolic extract (SEE), and sage hexane extract (SHE), the TPC, TFC, and TPAC values were 34.22 ± 0.70, 36.35 ± 0.83, 8.57 ± 0.53, 23.45 ± 0.83, 25.19 ± 0.71, 4.15 ± 0.31, 13.45 ± 0.64, 15.22 ± 0.67, and 2.65 ± 0.25 respectively (**Figure 1 A,B,C**).

The findings of our study do not resemble those of a more recent study by **El-Rafie *et al.*, (2023)**, who assessed the TPC and TFC in a hydroethanolic extract of *Salvia officinalis* leaves and found that their values were 236.91 ± 2.15 and 91.38 ± 0.97, respectively. Differences in the phenolic and flavonoid contents of *S. officinalis* extracts between our findings and those of **El-Rafie *et al.*, (2023)**, may be related to the plant's origin, the time of harvest, and the solvent used **(Duletić-laušević *et al.*, 2016)**. Numerous recent studies concluded that factors such as the plant part used for extraction, time of

sampling, choice of the extraction solvent, extraction techniques, and genetic and environmental (climate, location, temperature, fertility, pests and diseases) factors, could contribute to variations in the yield of total phenolics and flavonoids (**Ben Farhat *et al*., 2013; Orhan *et al*., 2013 and Martins *et al*., 2015**).

Sage polyphenols come in a wide variety of shapes, from very basic molecules to complex polymers, which are often identified by their flavonoids and phenolic acid content (**Dragovic-Uzelac *et al*., 2012**). According to studies, the leaves include phenolic acids such as rosmarinic, caffeic, and labiatic as well as carnosolic acid, flavonoids such as salvigenin, genkwanin, hispidulin, and luteolin and its derivatives (**Shamnas *et al*., 2014**).

The total carotenoid content in *Xanthophyllomyces dendrorhous*

Carotenoids are a type of pigment that is crucial in reducing oxidative reactions. They are strong antioxidants that may neutralize peroxyl and mono-molecular oxygen radicals. They stimulate redox sensitive regulatory pathways and affect cellular signaling (**Stahl and Sies, 2005**). Carotenoids are frequently found in high concentrations in plants, algae, and microorganisms. As neither humans nor animals can synthesise these carotenoids, it is essential to include them in the diet (**Sandmann, 1994**). Carotenoids act by inhibiting chain reaction by taking out free radicals, as these carotenoids contain polyene chain and long double bonds they perform as potent antioxidants (**Singh *et al*., 2021**).

The total carotenoid content in the *Xanthophyllomyces dendrorhous* culture broth was determined in the current study and was found to be 48.53 ± 3.28 µg/g of yeast (**Table 1**). In a related study, **Kalyanee *et al*., (2007)** found that *X. dendrorhous* GM807 and *X. dendrorhous* n485 produced 105 and 70 µg/g of total carotenoid respectively. The differences between the results of the current study and those of **Kalyanee *et al*., (2007)** in the total carotenoid contents could be explained by differences in the operating system parameters and the culture medium's composition.

Many studies have shown that the composition of the culture medium and the operating system parameters affect the amount of carotenoids that can be produced by *Xanthophyllomyces dendrorhous*. (**Liu and Wu., 2007; and Wang *et al*., 2019**)

Antioxidant and anti-inflammatory activities of sage leaves extracts (SME, SEE and SHE) and *Xanthophyllomyces dendrorhous* cultural filtrate.

Free radicals are molecules with one or more unpaired electrons that give them a significant amount of chemical reactivity. Many of these free radicals are produced by intracellular and extracellular biological fluid activities. Antioxidants act as a shield against free radicals, preventing any potential negative consequences that may result from the generation of these radicals. Antioxidants therefore prevent the reaction of free radicals (**Lobo *et al*., 2010 and Bolarin *et al*., 2016**).

Antioxidants are "free-radical scavengers" that can prevent delayed cell

damage from free radicals, which the body produces in response to environmental and other stresses. Free radicals can harm cells in a variety of ways. Carotenoids and phenolic compounds are thought to be a part of the antioxidant defense mechanism in both plants and animals (**Shahihi and Zhong, 2015**).

Hence, the current investigation concentrated on the antioxidant and anti-inflammatory effects of three sage leaves extracts (SME, SEE, and SHE) and *X. dendrorhous* culture filtrate. The antioxidant activity of several sage leaves extracts, and the *X. dendrorhous* culture filtrate was evaluated by scavenging hydrogen peroxide; the results are depicted in **Figure 2** as a percentage of H_2O_2 scavenged. The anti-inflammatory effects of various sage leaves extracts and *X. dendrorhous* cultural filtrate were assessed using the inhibition of albumin denaturation, with the results shown in **Figure 3** as a percentage inhibition of albumin denaturation.

As demonstrated in **Figure 2**, XDCF has the highest H_2O_2 scavenging activity (85.22% ± 1.28) followed by SEE (82.62% ± 1.98), SME (46.95% ± 0.89) and SHE (27.33% ± 0.96) respectively. Additionally, as shown in **Figure 3**, XDCF also showed the strongest anti-inflammatory efficacy, in the same pattern as scavenging hydrogen peroxide, with a percent reduction in albumin denaturation of (70.45% ± 1.98). SHE inhibited albumin denaturation to a minor degree (18.55 ± 0.89) while SME and SEE inhibited it to moderate degrees (48.55% ± 0.80 and 67.35 ± 1.32).

Phenolic compounds have been discovered to exert a variety of effects in recent years, including antioxidant (**Martins *et al.*, 2016**), antimicrobial (**Karunakaran *et al.*, 2018**), anticarcinogenic (**Muller *et al.*, 2019**), and anti-inflammatory (**Boo, 2019**) properties. They have also been found to prevent oxidative stress-related disorders such as diabetes, cancer, and cardiovascular disease (**Yasir *et al.*, 2016).**

One of the best and most natural sources of antioxidants is the sage plant. This characteristic is directly related to its medical value (**Loizzo *et al.*, 2014 and Medjahed *et al.*, 2016**). The high and varied biological activity of sage plants and their antioxidant properties are frequently attributed to phenolic substances. Plant extracts' chemical components and biological properties are greatly influenced by a wide range of abiotic variables, with temperature, precipitation, and season playing the largest roles (**Generalic *et al.*, 2011**).

Natural sources of carotenoids have recently gained prominence on the market as they possess a variety of biological characteristics, including the ability to defend against oxidative damage and the potential to serve as a precursor to vitamin A synthesis, The three most prevalent carotenoids are lycopene, β-carotene, and astaxanthin, with astaxanthin being particularly valuable for biotechnology (**Avalos and Limon., 2015**). A member of the xanthophyll family of carotenoid pigments, astaxanthin is a potent antioxidant. It is one of the primary pigments found in marine habitats, where it affects the color of microalgae, salmonid meat, some birds' plumage, and

crab shells (**Miao *et al.*, 2006 and Ytrestoyl and Bjerkeng, 2007**). It can be found naturally non-esterified or esterified with other fatty acids, such as palmitic acid, stearic acid, or linoleic acid, and has a hydrophobic nature (**Chávez-Cabrera *et al.*, 2010**). The non-esterified form is frequently degraded by oxidation (**Xin-Yi *et al.*, 2019**). Astaxanthin, one of the most potent carotenoids, is the primary product of *X. dendrorhous*. It has been used as an antioxidant, anti-inflammatory, immunological stimulant, and to boost the immune system and reproductive health in cattle. It has the ability to prevent oxidative damage to lipids, membrane lipoproteins, and cells (**Naguib, 2000**).

In the current experiment, the SEE showed the highest TPC, TFC, and TPAC values. Moreover, SEE and XDCF demonstrated the strongest anti-inflammatory and antioxidant capabilities. Thus, both formulations have been used against DMF toxicity in experimental animals.

The remedial impacts of SEE and XDCF against DMF toxicity on physiological responses in mice.

Indicators of the growth performance

The impacts of DMF, SEE, and XDCF on the growth parameters of males and females mice were shown in **Tables 2** and **3**, respectively. In comparison to the control group, the feed intake of males received DMF, SEE, DMF + SEE, and DMF + XDCF significantly $p \leq 0.05$ increased by 17, 15.14, 19.88, and 28.11%, respectively (**Tables 2**). Whereas XDCF, DMF + SEE and DMF + XDCF administrations significantly $p \leq 0.05$ reduced the body weight gain and average daily gain of females mice by 75.46, 62.28, 60.34, 76.48, 64.71, and 64.71 % respectively in comparison to the control group. Furthermore, compared to the control group, the feed efficiency of all treated female groups was significantly $p \leq 0.05$ depleted (**Table 3**).

N,N-dimethylformamide (DMF) is known to have harmful consequences, according to a number of studies. One of these studies was conducted by **Kennedy and Sherman's (1986)**. The findings from our investigation are in alignment with those of **Kennedy and Sherman's (1986)** study, who fed groups of weanling CD rats diets containing 0, 200, 1000, or 5000 ppm of dimethylformamide for 94 days. They concluded that overall body weight gain was lower than controls in high-dose males and females, respectively, mainly because average weight gain reductions in the males during weeks 1–5, and in the females during weeks 1-2. In high-dose males during weeks 1–5, and high-dose females during weeks 1-2, there was a reduction in food efficiency.

The relative weight of various organs

The relative weights of several organs, including the liver, kidney, spleen, heart, lung, and brain, were determined after the experiment had been performed for 42 days. The results are displayed in **Figures 4, 5, 6, 7, 8, and 9.** According to **Figure 4**, after receiving DMF for 21 days and going untreated for an additional 21 days, the liver percentage of the mice significantly ($p \leq 0.05$) increased in both the males and females by 27.81 and 28.99%,

respectively. The liver percentage of males and females which received SEE and XDCF after being exposed to DMF for 21 days, did not significantly ($p \leq 0.05$) differ from the control group. Also, the liver percentages of the SEE and XDCF treated groups did not significantly ($p \leq 0.05$) differ from the control group, demonstrating that these preparations didn’t have an adverse impact on the liver (**Figure 4**). The kidney percentage displayed a similar pattern to the liver percentage, but the XDCF had more benefits on improving the kidney percentage than the SEE preparation (**Figure 5).** Males exposed to 100 and 400 ppm and females exposed to 400 ppm in a similar study conducted by **Malley *et al.*, 1994**, had larger liver weights in relation to body weight. In another study, mice were treated with DMF at doses of 25, 100, and 400 ppm as part of a study on chronic exposure toxicity. The weight of the liver rose in mice at all tested doses **(OECD SIDS, 2001**).

In contrast, the DMF treatment significantly ($p \leq 0.05$) reduced the spleen percentage in both males and females, by 14.86 and 34.79%, respectively. The males and females that received SEE and XDCF after being exposed to DMF for 21 days, recorded spleen percentage values that have been close to those in the control group (**Figure 6**). Moreover, the DMF administration for 21 days caused an increase in heart percentage in both males and females by 44.62 and 14.64%, respectively. The heart percentage values of the male mice in the DMF + SEE and DMF + XDCF administrative groups were close to those in the control group, while those of the female mice in the DMF + XDCF administrative group were close to those in the control group (**Figure 7**). In addition, DMF administration for 21 days resulted in significant ($p \leq 0.05$) reductions in lung relative weight by 17.95 and 48.98% in male and female mice, respectively in comparison to the control values. After 21 days of exposure to DMF, SEE administration in males restored the relative weight of the closed lung to that of the control group, whereas XDCF administration in females restored the relative weight of the closed lung to that of the control group (**Figure 8**). No significant ($p \leq 0.05$) differences in the relative weight of the brain were found in any of the administered male and female groups (**Figure 9**).

Salvia officinalis extracts showed ameliorating impacts against the toxicity by several toxic compounds in numerous investigations. *Salvia officinalis* extract appears to have hepatoprotective properties against INH-induced liver damage in rats, according to the findings of a study by **Shahrzad *et al.*, (2014)**. According to this study, the antioxidant and free radical-scavenging properties of the flavonoids found in the extract may be the mechanism by which this activity occurs. Moreover, in the study of **Samiei *et al.*, 2020** the rats' renal toxicity caused by deltamethrin was significantly lessened by the *salvia officinalis* extract. The absolute and relative liver and kidney weights were restored to control values in the study by **Koubaa-Ghorbel *et al.*, 2020** using *Salvia officinalis* oil in mice receiving a high-fat diet.

The remedial impacts of SEE and XDCF against DMF toxicity on hematological and immunological responses in mice.

The detrimental impact of dangerous compounds on an animal's blood components can be identified using hematological markers. This could be impacted by interactions between toxic metabolites and biological components (**Abd El-Rahman *et al.*, 2017**). RBCs, Hb%, PCV%, MCV, MCH, and MCHC are recognized as good hematological markers of animal physiological condition and are helpful in toxicity monitoring (**Khan and Zafar, 2005**). The DMF treatment stimulates changes in several hematological parameters in DMF-intoxicated mice, as shown in **Table 4**. When compared to the control group, the RBCs, Hb%, and PCV% of chronically DMF-toxic male and female mice decreased significantly ($P \leq 0.05$) by 19.54, 29.07, 24.14, 21.78, 18.64, and 12.60%, respectively. After being exposed to DMF, the administration of SEE and XDCF reversed the alterations in RBCs, Hb, and PCV, and the recorded values were extremely near to the control values. There was no significant ($P \leq 0.05$) difference between the administration by SEE and XDCF and the control group for the RBCs, Hb and PCV%, and neither of these treatments significantly altered these parameters (**Table 4**). Also, after 42 days of the experiment, DMF administration significantly ($P \leq 0.05$) reduced MCV in female mice by 8.03% in comparison to the control group (Table 7). However, when DMF was administered to female mice, MCHC significantly ($P \leq 0.05$) rose by 15.81 compared to the control group (**Table 5**). The administration of SEE and XDCF restored the changes in MCV and MCHC after exposure to DMF, and the observed values were very close to the control values. At the end of the experimental period, there were no significant ($P < 0.05$) differences in MCH values in any of the treatment groups (**Table 5**).

Red cell volume distribution width (RDW) values are represented using the coefficient of variation of red cell volume distribution width (RDW-CV) and the standard deviation of red cell volume distribution width (RDW-SD) levels. The RDW-SD measures the width of the red cell distribution curve in femtoliters (fL). The RDW-CV is a calculation that determines the size of the mean cell as well as the width of the distribution curve. The indices are combined with the RDW to describe a population of RBCs (MCV, MCH, and MCHC). Hence, RDW-SD and RDW-CV were estimated in this investigation, and the findings are displayed in **Table 6**. According to the findings, after 42 days of the study period, DMF administration on both male and female mice significantly decreased ($P \leq 0.05$) RDW-SD and RDW-CV by 15.32, 26, 28.68, and 26.91%, respectively. After DMF exposure, SEE oral administration restored the RDW-SD and RDW-CV in male mice to control values (**Table 6**).

Bone marrow produces platelets, also referred to as thrombocytes, which are the second most prevalent blood cells. They move through the bloodstream and support hemostasis and wound recovery (**Pogorzelska *et al.*, 2020**). The most often assessed platelet markers are the mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), and platelet-large cell ratio (P-LCR) (**Budak *et al.*,**

2016). The size of platelets in the blood, or mean platelet volume (MPV), is the platelet characteristic that is most extensively studied (**Demirin** ***et al.*****, 2011**). The size distribution of the platelets produced by megakaryocytes is defined by the platelet distribution width (PDW), which increases when platelets are activated (**Osselaer** ***et al.*****, 1997**).

A measurement of total platelet mass expressed as a proportion of blood volume occupied is called plateletcrit (PCT) (**Budak** ***et al.*****, 2016**). One measure of platelet activity is the platelet larger cell ratio (P-LCR), which is a proportion of all blood platelets with a volume higher than 12 fL. (**Hong** ***et al.*****, 2014**). The findings of the evaluation of platelet counts and associated indices are shown in **Tables 7** and **8**. The collected results showed no significant ($P \leq 0.05$) differences in MPV values between any of the administered groups. DMF intraperitoneally injection in male mice significantly ($P \leq 0.05$) raised PLT and PDW by 51.25 and 32.64%, respectively. In contrast, the same treatment significantly ($P \leq 0.05$) reduced PLT in female mice by 49.92 %. Administration of SEE and XDCF following exposure to DMF returned the PDW levels in male mice to control values (**Table 7**).

Moreover, following DMF administration, the PCT and P-LCR values were significantly ($P \leq 0.05$) elevated in male mice and significantly ($P \leq 0.05$) decreased in female mice by 57.15, 53.20, 41.99, and 25.51% respectively. The PCT and P-LCR are restored to control levels in male mice when SEE was administered following DMF exposure. Similarly, the PCT values in female mice were returned to normal values following the administration of both SEE and XDCF preparations (**Tables 8**).

White blood cells (leukocytes), which defend the body from antigen invasion, are crucial for immunological function. They are divided into groups based on the type of granules in their cytoplasm and the shape of their nuclei. As a result, granulocytes and agranulocytes are the two categories of white blood cells. Neutrophils, eosinophils, and basophils are types of granulocytes, whereas lymphocytes and monocytes, which can undergo multiple cycles of activity before dying, are types of agranulocytes (**Junqueira** ***et al.*****, 1992**). According to **Klein and Horej (1997)**, lymphocytes help in the recognition of a variety of antigens, differentiation and maturation to functional capability, antigen response, and immunologic memory. Neutrophils and monocytes both exhibit phagocytic activity. They assault and eliminate bacteria, waste products from cells, and foreign particles (**Dacie and Lewis, 1991**).

As a result, the amount of white blood cells, lymphocyte percentage, and neutrophil percentage were evaluated. The results are shown in **Figures 10, 11,** and **12**. Male and female mice exposed to DMF had lower white blood cell counts than the control group by 60.43 and 51.12%, respectively. White blood cell counts in female mice exposed to DMF were returned to normal by an intraperitoneal injection of XDCF (**Figure 10**). No significant ($P \leq 0.05$) variations in lymphocyte percentage can be seen among all administered groups (**Figure 11**). Also, the DMF intraperitoneal injection significantly ($P \leq 0.05$) reduced the neutrophil

percentage in female mice by 30.59%. Moreover, the neutrophil percentage was not altered by the intraperitoneal injection of XDCF to return it to normal levels in female mice **(Figure 12)**.

In various pervious investigations, dimethyl formamide had an effect on a range of hematological parameters, including increases in lymphocyte percentage **(Major *et al*., 1998)**, decreases in hematocrit, and levels of hemoglobin, **(Craig *et al*., 1984)** which is consistent with our findings. In Ehrlich ascites carcinoma (EAC)-bearing mice, sage extracts showed improvement in the hematological parameters, including an increase in RBCs, hemoglobin, and platelets count **(Al-Motwaa *et al*., 2019)**.

Astaxanthin is the main carotenoid pigment produced by *Xanthophyllomyces dendrorhous*. There is limited information on the physiological effects of either purified astaxanthin or astaxanthin produced from red yeast on hematological parameters. Red blood cells (RBCs) were unaffected by the addition of astaxanthin from red yeast to broiler diets, according to **Jeong and Kim (2014)** study. Additionally, **Cao and Wang (2014)** emphasized that, astaxanthin supplementation for 35 days increased RBCs, but had no influence on mean corpuscular hemoglobin (MCH) or volume (MCV) values, while significantly decreased hematocrit and hemoglobin.

CONCLUSION

In the current investigation, SEE and XDCF reduced the toxicity of dimethyl formamide on physiological, hematological, and immunological parameters in mice. The antitoxic properties noticed in this study could be explained by the presence of numerous phytochemicals in SEE, including polyphenols, flavonoids, phenolic acids, and others as well as carotenoids in XDCF. Finally, the results show that SEE and XDCF can be used as antitoxic agents and have several positive health effects.

Table (1): The total carotenoid content in *Xanthophyllomyces dendrorhous* grown in YM broth media for 10 days at 22°C

Strain	Total carotenoid content (µg/g of yeast) ± SEM
Xanthophyllomyces dendrorhous NRRL Y-17269	48.53 ± 3.28

Table (2): The growth parameters of male mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days.

Treatment Groups	Indices					
	Initial weight, g	Final weight, g	Body weight gain, g	Average daily gain, g	Feed intake g/day	Feed efficiency (%)
Control	27.59±1.24	35.36±0.72	7.77±1.58	0.18±0.03	11.33±0.86[a]	1.58±0.39[ab]
DMF	29.36±2.18	36.75±1.03	7.39±2.40	0.17±0.05	13.65±0.71[ab]	1.24±0.41[ab]
SEE	26.90±0.29	34.55±1.27	7.65±1.49	0.18±0.03	13.35±0.48[ab]	1.34±0.21[ab]
XDCF	29.50±1.43	41.19±2.34	11.69±1.46	0.27±0.03	11.79±0.64[a]	2.29±0.35[b]
DMF + SEE	32.13±0.38	40.13±0.55	8.00±0.90	0.19±0.02	14.14±1.38[ab]	1.34±0.20[ab]
DMF + XDCF	31.62±0.49	38.52±0.51	6.90±0.87	0.16±0.02	15.76±1.84[b]	1.01±0.25[a]

Values are presented as mean ± SEM, Values in each column which have different superscript letters are significantly different ($p \le 0.05$). DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate.

Table (3): The growth parameters of female mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days.

Treatment Groups	Indices					
	Initial weight, g	Final weight, g	Body weight gain, g	Average daily gain, g	Feed intake g/day	Feed efficiency (%)
Control	28.31±1.85	35.52±0.71	7.21±1.36^{b}	0.17±0.03^{b}	9.41±0.83^{a}	1.80±0.34^{b}
DMF	32.14±1.30	36.89±0.80	4.75±0.96^{ab}	0.11±0.02^{ab}	15.11±1.34^{c}	0.72±0.17^{a}
SEE	31.08±0.95	34.45±1.34	3.37±1.71^{b}	0.08±0.04^{ab}	12.81±0.75^{bc}	0.62±0.35^{a}
XDCF	38.96±1.70	40.73±1.58	1.77±0.77^{a}	0.04±0.01^{a}	10.12±0.50^{ab}	0.39±0.19^{a}
DMF + SEE	36.78±0.87	39.50±0.99	2.72±0.31^{a}	0.06±0.02^{a}	14.10±1.47^{c}	0.42±0.08^{a}
DMF + XDCF	32.75±1.63	35.61±0.53	2.86±1.34^{a}	0.06±0.03^{a}	12.14±0.71^{abc}	0.49±0.08^{a}

Values are presented as mean ± SEM, Values in each column which have different superscript letters are significantly different ($p \le 0.05$). DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate

Table (4): The RBCs, PCV and Hb of males and females mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days.

Treatment Groups	RBC's (10^6/µL)		PCV (%)		Hb (g/dL)	
	Males	Females	Males	Females	Males	Females
Control	6.50±0.16^{b}	6.64±0.36^{bc}	34.80±1.03^{c}	37.20±0.69^{c}	14.70±1.03^{b}	12.70±0.46^{abc}
DMF	5.23±0.32^{a}	4.71±0.18^{a}	26.40±1.44^{a}	29.10±0.63^{a}	11.96±0.86^{a}	11.10±0.46^{a}
SEE	6.30±0.10^{b}	6.67±0.33^{bc}	34.55±0.37^{c}	37.20±1.09^{c}	13.80±0.63^{ab}	14.30±0.56^{cd}
XDCF	5.60±0.28^{a}	6.82±0.19^{c}	29.70±0.86^{b}	37.30±0.63^{c}	12.60±0.46^{ab}	14.80±0.63^{d}
DMF + SEE	6.40±0.10^{b}	5.95±0.18^{b}	35.40±0.78^{c}	32.25±0.77^{b}	15.10±1.27^{b}	12.60±0.51^{ab}
DMF + XDCF	6.21±0.10^{b}	6.17±0.06b^{c}	34.70±1.32^{c}	32.60±0.05^{b}	14.20±0.75^{ab}	13.20±0.17^{bcd}

Values are presented as mean ± SEM, Values in each column which have different superscript letters are significantly different ($p < 0.05$). RBC's = red blood cells; PCV = peaked cell volume; Hb = hemoglobin, DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate

Table (5): The MCV, MCH and MCHC of males and females mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days.

Treatment Groups	MCV (fL)		MCH (pg)		MCHC (g/dl)	
	Males	Females	Males	Females	Males	Females
Control	53.60±0.69	56.10±1.03^{c}	22.60±1.50^{a}	19.10±0.34^{a}	42.20±0.75^{ab}	34.10±0.98^{a}
DMF	54.50±0.86^{a}	51.60±0.46^{a}	22.50±0.86^{a}	20.80±0.46^{ab}	41.10±0.63^{ab}	40.50±0.69^{b}
SEE	59.20±1.61^{b}	55.90±0.75^{c}	23.70±0.75^{a}	21.40±1.29^{ab}	43.03±0.12^{b}	36.73±1.34^{ab}
XDCF	53.10±0.63^{a}	54.80±0.69^{bc}	22.50±0.28^{a}	21.70±0.79^{b}	42.40±0.86^{ab}	39.60±0.43^{b}
DMF + SEE	52.80±0.46^{a}	54.25±0.37^{bc}	21.70±1.27^{a}	21.10±0.23^{ab}	39.90±0.63^{a}	38.95±0.66^{b}
DMF + XDCF	55.90±0.98^{a}	52.75±0.60^{ab}	22.80±0.63^{a}	21.40±0.51^{ab}	40.90±1.61^{ab}	40.55±0.54^{b}

Values are presented as mean ± SEM, Values in each column which have different superscript letters are significantly different ($p < 0.05$). MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration, DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* Cultural filtrate

Table (6): The RDW-SD and RDW-cv of males and females mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days.

Treatment Groups	RDW-SD (fL)		RDW-CV (%)	
	Males	Females	Males	Females
Control	29.70±0.40^{ab}	42.70±1.44^{c}	14.30±0.63^{bc}	19.70±0.69^{c}
DMF	25.15±0.56^{a}	31.60±0.92^{a}	10.20±0.40^{a}	14.40±0.71^{a}
SEE	28.80±0.43^{a}	37.20±0.69^{b}	13.80±0.69^{b}	17.20±0.28^{b}
XDCF	37.20±0.38^{bc}	33.40±1.09^{a}	18.10±0.95^{d}	15.70±0.56^{ab}
DMF + SEE	28.40±0.31^{a}	34.35±0.54^{ab}	14.10±0.51^{bc}	16.35±0.14^{b}
DMF + XDCF	41.30±0.98^{c}	32.50±0.51^{a}	16.30±0.86^{cd}	15.90±0.57^{ab}

Values are presented as mean ± SEM, Values in each column which have different superscript letters are significantly different ($p < 0.05$). RDW-SD = standard deviation of red blood cell distribution width, RDW-CV = coefficient of variation of red blood cell distribution width;, DMF = Dimethyl formamide , SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate

Table (7): The PLT, MPV and PDW of males and females mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days.

Treatment Groups	PLT (10^3/µL)		MPV (fL)		PDW %	
	Males	Females	Males	Females	Males	Females
Control	176±3.46[a]	605.00±2.88[c]	7.30±0.23[ab]	7.90±0.34[a]	8.86±0.43[ab]	10.50±0.40[ab]
DMF	361±3.46[d]	303.00±3.46[b]	7.90±0.40[ab]	7.30±0.23[a]	13.10±0.23[c]	9.70±0.23[a]
SEE	185±2.88[ab]	280.00±5.19[b]	6.93±0.43[a]	7.67±0.26[a]	7.86±0.31[a]	8.86±0.49[a]
XDCF	351±4.04[cd]	205.00±6.35[a]	7.40±0.23[ab]	7.80±0.40[a]	8.40±0.17[a]	12.00±0.15[b]
DMF + SEE	193±4.44[b]	633.33±15.87[d]	7.60±0.23[ab]	7.30±0.15[a]	7.90±0.11[a]	9.45±0.31[a]
DMF + XDCF	345±3.58[c]	630.00±9.36[d]	8.10±0.48[b]	7.40±0.15[a]	10.00±0.86[b]	10.45±0.14[ab]

Values are presented as mean ± SEM, Values in each column which have different superscript letters are significantly different ($p < 0.05$). PLT= platelets; MPV = mean platelet volume, PDW =platelet distribution width; DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* Cultural filtrate

Table (8): The PCT and P-LCR of males and females mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days.

Treatment Groups	PCT (%)		P-LCR (%)	
	Males	Females	Males	Females
Control	0.12±0.01[a]	0.47±0.04[b]	9.30±0.34[a]	14.90±0.80[b]
DMF	0.28±0.03[b]	0.22±0.04[a]	16.03±0.72[b]	11.10±0.34[a]
SEE	0.14±0.08[a]	0.21±0.04[a]	8.45±0.25[a]	12.90±0.41[ab]
XDCF	0.25±0.03[b]	0.47±0.05[b]	8.30±0.40[a]	14.60±0.46[b]
DMF + SEE	0.13±0.05[a]	0.38±0.03[b]	8.48±0.22[a]	10.70±0.90[a]
DMF + XDCF	0.52±0.02[c]	0.35±0.06[ab]	17.20±0.20[b]	11.70±0.34[a]

Values are presented as mean ± SEM, Values in each column which have different superscript letters are significantly different ($p < 0.05$). PCT = procalcitonin; P-LCR = platelet large cell ratio; DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate

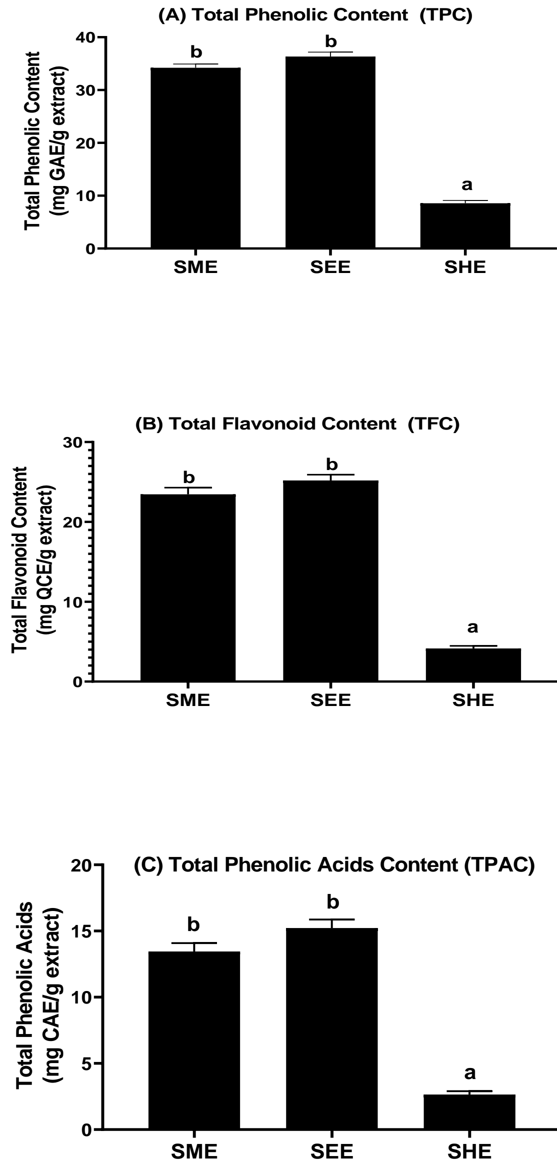

Figure (1): The total phenolic content (mgGAE/g dried extract), total flavonoid content (mgQCE /g dried extract) and total phenolic acids content (TPAC) (mgCAE/g dried extract) in various sage leaves extracts. SME = Sage methanolic extract, SEE = Sage ethanolic extract, SHE = Sage hexane extract. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

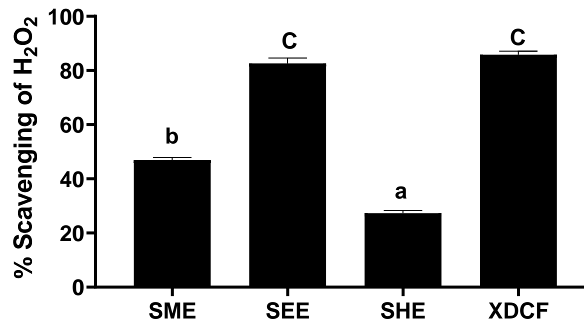

Figure (2): The antioxidant activity (% Scavenging of H_2O_2) of various Sage leaves extracts and *Xanthophyllomyces dendrorhous* cultural filtrate. SME = Sage methanolic extract, SEE = Sage ethanolic extract, SHE = Sage hexane extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

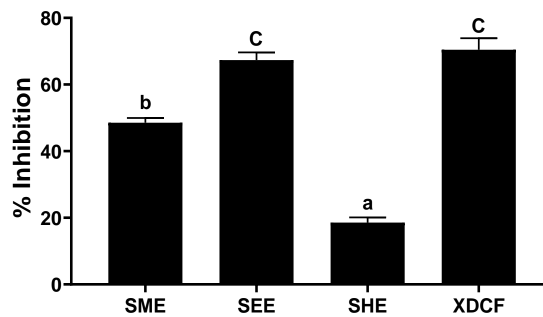

Figure (3): The anti-inflammatory activity (% Inhibition of albumin denaturation) of various Sage leaves extracts and *Xanthophyllomyces dendrorhous* cultural filtrate. SME = Sage methanolic extract, SEE = Sage ethanolic extract, SHE = Sage hexane extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

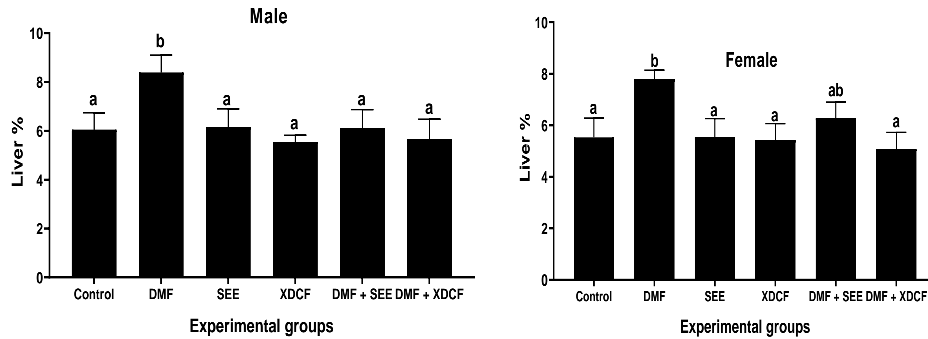

Figure (4): The relative weight of liver in male and female mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days. DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

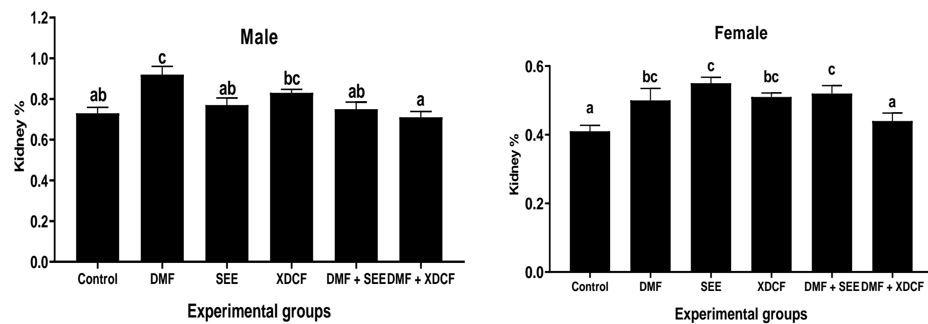

Figure (5): The relative weight of kidney in male and female mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days. DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

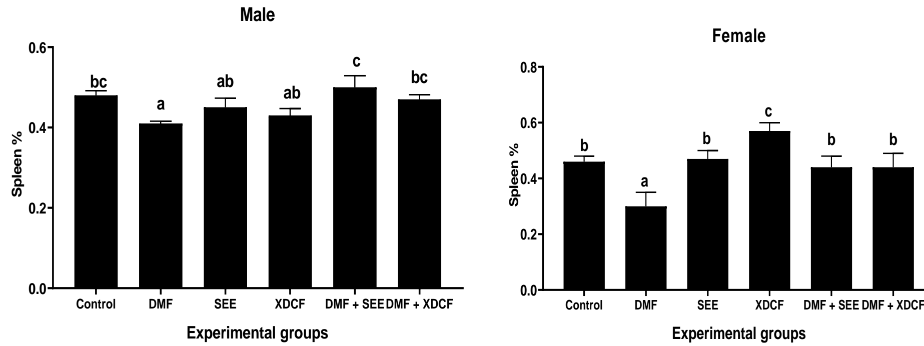

Figure (6): The relative weight of spleen in male and female mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days. DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

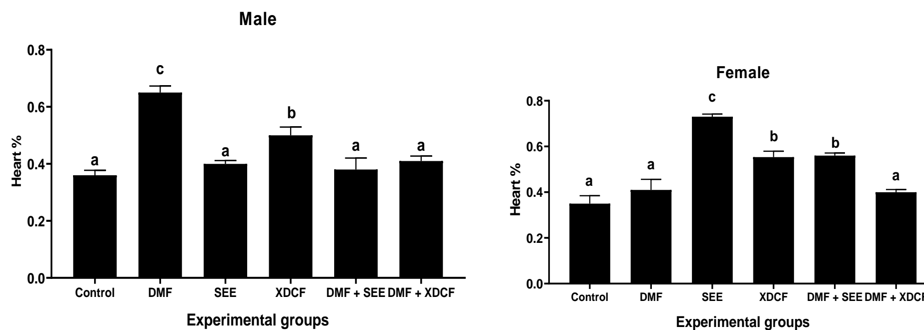

Figure (7): The relative weight of heart in male and female mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days. DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

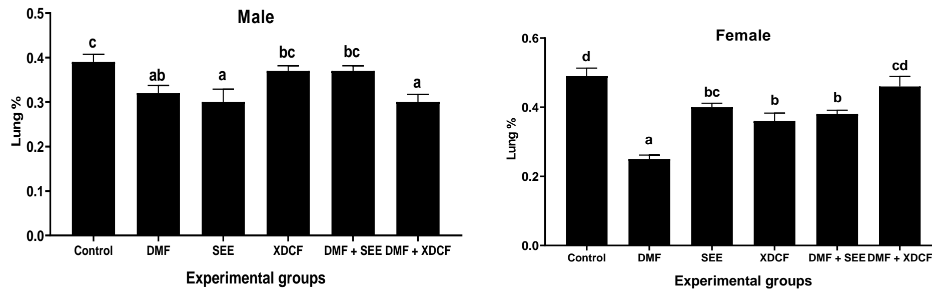

Figure (8): The relative weight of lung in male and female mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days. DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

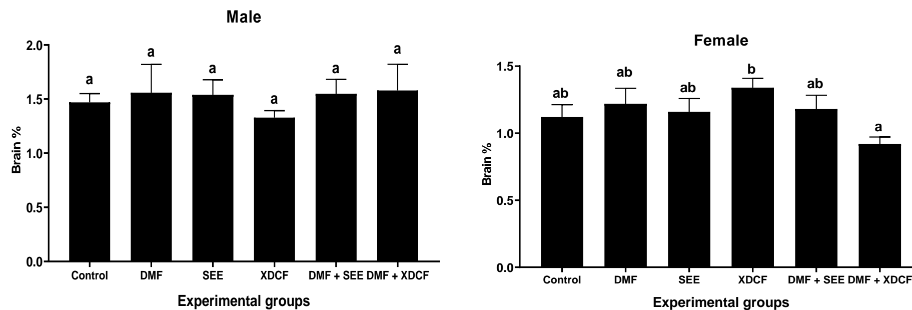

Figure (9): The relative weight of brain in male and female mice exposed to dimethyl formamide, Sage ethanolic extract and *Xanthophyllomyces dendrorhous* cultural filtrate for 42 days. DMF = Dimethyl formamide, SEE = Sage ethanolic extract, XDCF = *Xanthophyllomyces dendrorhous* cultural filtrate. Values are presented as mean ± SEM. Values with various superscript letters differ significantly ($p \leq 0.05$).

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الملخص العربي

الآثار المحتملة لمستخلص أوراق المرمرية وراشح المزرعة الفطرية للخميرة الحمراء علي الاستجابات الفسيولوجية والسمية الدموية المستحثة بثنائي ميثيل فورماميد في الفئران

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جمهورية مصر العربية

هدفت هذه الدراسة لتقييم الآثار المحتملة للمستخلص الإيثانولي لأوراق المرمرية وراشح المزرعة الفطرية للخميرة الحمراء علي الاستجابات الفسيولوجية والهيماتولوجية في الفئران المحقنونه في الغشاء البرتوني للبطن بمركب ثنائي ميثيل فورماميد. تم تقسيم ٣٦ فأر من ذكور وإناث الفئران السويسرية البيضاء الي ١٢ مجموعة (٦ مجموعات ذكور , ٦ مجموعات اناث) واحتوت كل مجموعة علي ٣ فئران. وكانت المعاملات التجريبية لكل من مجموعات الذكور والاناث متشابهة. استخدمت المجموعة الأولي كمجموعة ضابطة. تم معاملة المجموعة الثانية والثالثة والرابعة بمركب ثنائي ميثيل فورماميد بتركيز ٣٧٨ ملليجرام / كجم وزن جسم عن طريق الحقن في الغشاء البروتوني للبطن ، المستخلص الايثانولي لأوراق المرمرية بتركيز ١٠٠ ملليجرام / كجم وزن جسم يوميا عن طريق الفم ، براشح المزرعة الفطرية للخميرة الحمراء بتركيز ٣٣٠٠ ميكروليتر / كجم وزن جسم يوميا عن طريق الحقن في الغشاء البروتوني للبطن على التوالي يوميا لمدة ٢١ يوم. في حين أن المجموعة الخامسة تمت معاملتها بمركب ثنائي ميثيل فورماميد بتركيز ٣٧٨ ملليجرام / كجم وزن جسم يوميا عن طريق الحقن في الغشاء البروتوني للبطن لمدة ٢١ يوم ثم عقب ذلك تمت المعاملة بالمستخلص الايثانولي لأوراق المرمرية بتركيز ١٠٠ ملليجرام / كجم وزن جسم يوميا عن طريق الفم لمدة ٢١ يوم أخرى. أما المجموعة السادسة فعوملت بمركب ثنائي ميثيل فورماميد بتركيز ٣٧٨ ملليجرام / كجم وزن جسم يوميا عن طريق الحقن في الغشاء البروتوني للبطن لمدة ٢١ يوم ثم عقب ذلك تمت المعاملة براشح المزرعة الفطرية للخميرة الحمراء بتركيز ٣٣٠٠ ميكروليتر / كجم وزن جسم يوميا عن طريق الحقن في الغشاء البروتوني للبطن لمدة ٢١ يوم أخرى. أدت معاملة الفئران بمركب ثنائي ميثيل فورماميد الي زيادة الوزن النسبي للكبد والكلي والقلب وانخفاض الوزن النسبي للرئه والطحال في مجموعات الذكور والاناث مقارنة بالمجموعة الضابطة. أما عدد كرات الدم الحمراء، ونسبة الهيموجلوبين، وطول عمود الدم فقد انخفض بشكل معنوي نتيجة لمعاملة الفئران بمركب ثنائي ميثيل فورماميد. أدت معاملة الفئران بالمستخلص الإيثانولي لأوراق المرمرية وراشح المزرعة الفطرية للخميرة الحمراء إلى تحسين بعض مؤشرات أداء النمو، وتقليل التأثيرات الضارة لمركب ثنائي ميثيل فورماميد على بعض الأعضاء وقياسات الدم. وتؤكد نتائج هذه الدراسة أنه يمكن استخدام المستخلص الإيثانولي لأوراق المرمرية وراشح المزرعة الفطرية للخميرة الحمراء كعوامل مضاده للسموم بالإضافة الي انه لها مدي واسع من الفوائد الصحية.