

Protective Effect of Horsetail (*Equisetum arvense*, L.) Against Food Azo Dye Tartrazine toxicity on some biochemical parameters and antioxidants of Male Rats: Role of Oxidative Stress

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ABSTRACT

Background: Nowadays, there is a growing interest in medicinal plant usage. Horsetail (*Equisetum arvense*, L.) plant family *Equisetaceae* has many uses in traditional medicine and possesses several pharmacological effects, mostly antioxidant effects. Tartrazine (TZ) is an organic azo dyes widely used in coloring food additives, drugs and cosmetics. It can trigger oxidative stress which consequently generates metabolic disorders as hepatic and renal toxicity. Therefore, this study was designed to evaluate the protective properties of horsetail against TZ mediated oxidative stress in rats. **Material and methods:** forty-two rats were randomly classified into six groups (7 rats each). The first group (G1) kept as a negative control, the other five groups gave oral tartrazine-intoxicated, 300 mg /kg b.wt. /day. One group served as a positive control (G2), while the others TZ groups treated with horsetail powder and extract as follow: G3 10% Horsetail powder / kg/ diet/day, G4: 10 mg Horsetail extract /kg b.wt., G 5: 20% Horsetail powder / kg/ diet/day and G 6: 20 mg Horsetail extract /kg b.wt. **Results** showed that administration of Horsetail (powder and extract) at all dosages significantly improved, rats body weight gain percentage, HB, PCV, liver functions (ALT, AST and ALP) and kidney functions (creatinine and uric acid) as compared to TZ group. These were associated with significant increment of plasma antioxidants biomarkers GST, SOD and CAT and decrement of NO oxidative stress biomarker, also in liver homogenate tissues, Horsetail significantly increased GST, SOD, and GPX however, decreased MDA matched to TZ group. **Conclusion:** It could be concluded that horsetail (powder and extract) showed a promising protective role against adversely tartrazine affect and alteration biochemical markers in vital organs (liver and kidney) associated with decrease the oxidative stress. The mechanism may involve antioxidant effect and mitigation of lipid peroxidation. 11

Key words: Tartrazine, Horsetail (*Equisetum arvense*), Hemoglobin, Antioxidants, Oxidative Stress, renal and hepatic functions.

INTRODUCTION

Color is one of the main characteristics of food. When the natural color of food is lost during processing, synthetic colors can be added to enhance the attractiveness and zest of food. Currently, the health risks associated with exposure to food additives are receiving considerable attention from consumers, nutritionists, and toxicologists (**Abd-Elhakim et al., 2018A; B and C and Abo-El-Sooud et al., 2018 A and B**). Tartrazine (TZ) as a well-known sulfonated azo dye, is an orange-colored water-soluble powder extensively used to color food products, known as synthetic lemon yellow (**Elhakim et al., 2007** and **Sahnoun et al., 2018**). It is widely used as food additive which has been also applied in the drugs and cosmetics industries. It has been added to give a pleasant color to cake mixes, biscuits, jams, jellies, chewing gums, condiments, beverages, sauces, flavored chips and ice cream (**Mehedi et al., 2009; Kashanian and Zeidali, 2011; Vidal et al., 2018 and Bonciu et al., 2020**). Furthermore, in many developing countries it has been used as a substitute for saffron for cooking (**Mehedi et al., 2009**).

Tartrazine has been linked to the development of several disorders including asthma (**Arderm and Ram, 2001**), hyperactivity behavioral changes (**Bloom et al., 2016 and Oyewole and Oladele, 2016**), hypersensitivity reactions (**Leo et al., 2018**), neurotoxic (**Mohamed et al., 2015 and Yadav et al., 2019**), brain damage (**Hosieny et al., (2021)**), learning and memory defects (**Gao et al., 2011**), gastrointestinal and liver injury (**El Rabey et al., 2019 and Gijbels et al., 2021**), hormonal (**Abdel-Aziz et al., 2019**), endocrinal (**El-Sakhawy et al., 2019**), and teratogenic (**Hashem et al., 2019**) potentials.

The acceptable daily intake (ADI) for T is 7.5 mg/kg b.w. (**Tanaka et al., 2008 and Mpountoukas et al., 2010**). At ADI level, its consumption is safe as no dangerous effects have been recorded in either humans or experimental models (**Tanaka et al., 2008 and Poul et al., 2009**). Toxicokinetic studies showed that only 2% of the ingested tartrazine is directly absorbed and most tartrazine is broken down into smaller metabolites like sulfanilic

acid and aminopyrazolone in the colon (Elhkim *et al.*, 2007). Where the azo compounds, with the (-N=N-) functional group and aromatic rings linked to them, are reductively cleaved into aromatic amines. Some of these amines are toxic, carcinogenic and mutagenic (Bloom *et al.*, 2016; Chung, 2000; Zhang and Ma, 2013 and Rovina *et al.*, 2017). Moreover, these metabolites of tartrazine can generate reactive oxygen species (ROS), generating oxidative stress, decrease antioxidant defense mechanisms (Boussada *et al.*, 2017) and affect hepatic and renal architectures and biochemical profiles (Himri *et al.*, 2011). Gautam *et al.* (2010) found that TZ administered to mice lead to hepato-cellular damage, and biochemical and reproductive alterations in high doses, and even in low doses. TZ has been reported to alter the hepatic and renal parameters and induce oxidative stress by forming free radicals (Amin *et al.*, 2010 and Ali *et al.*, 2016). Mpountoukas *et al.* (2010) and Imane *et al.* (2012) indicated that TZ could potentially be genotoxic for human lymphocytes and could bind directly to DNA. Also, Abd-Elhakim *et al.*, (2018A) indicated that tartrazine exerts haematotoxic and immunotoxic effects following long-term exposure and induced significant anaemia and leukocytosis.

Horsetail (*Equisetum arvense*) belongs to the Equisetopsida family, it grows in several regions of in the temperate zones of the Northern Hemisphere (Asgarpanah and Roohi, 2012 and Hager, 2013). It has long been used in traditional medicine (Czygan and Wichtl, 1997; Madaus, 1990; Nagai *et al.*, 2005 and World Health Organization, 2010). *E. arvense* is known as a "liver herb" in the American and European marketplaces and is gaining popularity as a supplement for improving liver function, hyperlipidemia, and alcohol metabolism (Dos Santos *et al.*, 2005 and Kong, 2013). The putative medicinal properties are supported by a number of studies, which found hepatoprotective (Oh *et al.*, 2004), renoprotective (Boeing *et al.*, 2021 and Pechter *et al.*, 2018), diuretic (Wright *et al.*, 2007), anti-bacterial (Bessa Pereira *et al.*, 2012; Milovanović *et al.*, 2007 and Pallag *et al.*, 2018), anti-diabetes (Fajri *et al.*, 2020 and Revilla *et al.*, (2002),

anticancer (**Batir-Marin et al., 2021 B** and **Bhat et al., 2020**), antioxidant effects (**Batir-Marin et al., 2021 A and B**; **Cetojević-Simin et al., 2010** and **Wu et al., 2010**), modulates oxidative stress (**Pallag et al., 2018**) and antiproliferative properties (**Yamamoto et al., 2004**). Furthermore, anti-inflammatory properties for the treatment of wounds or inflammatory diseases such as arthritis have been described (**Asgharikhatooni et al., 2015**; **Briceño-Cardona et al., 2021**; **Costa-Rodrigues et al., 2012**; **Do Monte et al., 2004** and **Shiba et al., 2021**).

Field horsetail owes its healing effect to its chemical structure. Apart from the over 10% of inorganic substances (most of them are silicic acid and potassium salts), it contains mainly (**Mimica-Dukic et al., 2008**) phytosterols, alkaloids, tannins, triterpenoids (**Četojević-Simin et al., 2010**; **D'Agostino et al., 1984** and **Oniszczyk et al., 2014**), ascorbic acid (**Nagai et al., 2005**), phenolic acids (**Francescato et al., 2013**), polyunsaturated acids, rare dicarboxylic acids and styrylpyrones (**Beckert et al., 1997**), and flavonoids (**Pittler, 2010** and **Saleh et al., 1972**). Studies of *E. arvense* have reported on its antioxidant (**Ismail et al., 2020** and **Oh et al., 2004**). According to an in vitro experiment using HepG₂ cells, onitin and luteolin have liver-protective, superoxide-scavenging, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activities (**Oh et al., 2004**). Also, the relationship between the antioxidant activity and the content of phenolics in the horsetail extracts was identified (**Kim et al., 2016**; **Masłowski et al., 2020**; **Nunes et al., 2017**; **Belščak-Cvitanović et al., 2018** and **Patova et al., 2019**). Moreover horsetail is rich in many kinds of vitamins and trace elements. The high contents of these elements, high antioxidative activity this make horsetail is not only a health food, but also useful to protect against the various diseases (**Nagai et al., 2005** and **Oniszczyk et al., 2014**). The present work aims to evaluate the protective properties of horsetail against TZ toxicity, the effect on body-weight gain, hemoglobin, some biochemical parameters related to renal and hepatic functions and antioxidants / oxidative stress biomarkers in plasma and liver tissues.

MATERIALS AND METHODS

Materials:

- B-Tartrazine: was purchased from any local company for cosmetics, Cairo, Egypt.
- Horsetail was obtained from a local market in Cairo city, Egypt.
- Casein, vitamins, minerals and cellulose were obtained from El-Gomhariya Pharm. and Chem. Ind. Comp., Cairo, Egypt. While starch and corn oil were obtained from local market.
- Forty –two mature male albino rats of Sprague - Dawley strain weighing 110 ± 5 g were obtained from Laboratory of Animal Colony, Helwan, Egypt.

Methods:

Horsetail powder was added to the diet as 10, 20% of the diet. The other part was used for preparation of methanol extract. Horsetail powdered was soaked in 500 ml of 80% ethanol with frequent agitation. Clarification was then carried out using vacuum filtration through filter paper watman 2. The resultant extract was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40°C. The rat dose of Horsetail extract was 10, 20 mg/kg b.wt according to **Irkin and Korukluoglu (2017)**.

Design experimental animals:

All animals were kept under observation for five days before experiment, fed on standard diet according to **NRC (1995)** and water ad libitum. The standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg), cellulose (30 g/kg), corn oil (50g/kg), mineral mixture (100g/kg), vitamins mixture (20g/kg) and DL-methionine (3g/kg).

Rats were randomly classified into six groups (7 rats each). The first group kept as normal control fed standard diet only. The other five groups gave oral tartrazine-intoxicated, 300 mg /kg of body weight/day according to **El Golli et al. (2016)**. One group served as non-treated positive control while other groups treated with Horsetail powder and Extract as follow:

Group 1: normal control fed on the basal diet only.

Group2: positive control gave oral tartrazine-intoxicated, 300 mg /kg of body weight / day.

Group 3: positive control + 10% Horsetail powder / kg/ diet/day.

Group 4: positive control +10 mg Horsetail extract /kg b.wt.

Group 5: positive control +20% Horsetail powder / kg/ diet/day.

Group 6: positive control +20 mg Horsetail extract /kg b.wt.

The study was assigned for eight weeks. The food intake was calculated daily and the body weight gain was recorded weekly. Food and protein efficiency ratio (FER&PER) were calculated according to **Chapman *et al.* (1950)**.

Biochemical analysis:

At the end of the experiment, the rats were sacrificed to obtain blood samples. Heparinized blood was analyzed for estimation of hemoglobin (HB) and packed cell volume (PCV) according to **Drabkin (1949)** and **Mc Inory, (1954)**, respectively.

Determination of liver and kidney functions: Serum alanine and aspartate aminotransferase (ALT, AST), alkaline phosphates (AP) enzymes, creatinine and uric acid were estimated according to **Reitman and Frankel (1957)**, **Kind and King (1954)**, **Hare (1950)** and **Fossati, *et al.*, (1980)**, respectively.

Determination of antioxidant enzymes: Plasma glutathione transferase (GST), catalase, and superoxide dismutase enzymes (SOD) and nitric oxide (NO) were estimated according to **Habig (1974)**, **Claiborne (1985)**, **Beuchamp and Fridovich, (1971)** and **Green *et al.*, (1981)**, respectively.

liver of each rats were rapidly removed and perfuse with 50 to 100 of ice cold 0.9% NaCL solution for estimation of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST) and malondialdehyde (MDA) according to **Beuchamp and Fridovich (1971)**, **Weiss *et al.* (1980)**, **Ellman (1958)** and **Uchiyama and Mihara (1978)**, respectively.

Statistical analysis:

The obtained data were statistically analyzed using computerized SPSS. Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's

multiple range test and $p < 0.05$ was used to indicate significance between different groups (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Effect of tartrazine and horsetail (powder and extract) on final body weight (FBW), body weight gain (BWG), feed intake, feed efficiency ratio (FER), protein efficiency ratio (PER) of the experimental rats groups:

The initial body weights of rats were similar in all groups and all of them gave positive body weight gain at the end of the experiment. Meanwhile, the administration of TZ to rats significantly decreased FBW, BWG%, FER and PER compared to the negative control group and all treatment groups as shown in **Table (1)**. It was noticed that the treated rats with TZ+ horsetail extract were the best mitigating ability against TZ toxicity; although, all of horsetail (powder and extract) showed a positive and protective effect on TZ toxicity. These results are in accordance with **Amin *et al.* (2010)** and **El Desoky *et al.* (2017)** who reported that tartrazine produced a significant decrease in body-weight gain. Also, **Arefin *et al.* (2017)** revealed a highly noticeable decrease in the body weight gain of mice at 400mg/kg dose compared with the negative control group. The significantly losses in body mass of rats fed TZ might be due to TZ reducing the palatability of food or otherwise resulting in avoidance. Furthermore, TZ might result in generation of free radicals, which resulted in oxidative stress that caused metabolic disorders and general losses of body mass (**El Desoky *et al.*, 2017**). Body weight loss is considered by some authors to be a good reliable sensitive toxicity indicator (**Ezeuko *et al.*, 2007** and **Arefin *et al.*, 2017**). The result indicates the potentiality of tartrazine to alter the growth as a function of toxicity. However, **Tanaka (2006)** indicated that there were no significant effects of tartrazine on the average feed intake. **Gautam *et al.* (2010)** observed a significant increase in the body weight of TZ experimental groups when compared to control group. *E. arvense* extract oral ingestion significantly increased the FBW and BWG% at doses of 25, 50,

75 mg/kg matched to CCl₄ intoxicated rats (Ragheb and Alamri, 2020).

Table (1): Mean values \pm SD of final body weight (FBW), body weight gain (BWG), feed intake, feed efficiency ratio (FER) and protein efficiency ratio (PER) of the experimental rat groups.

Groups Variables	Normal control G1	Positive control G2	10% Horsetail powder G3	10mg Horsetail extract G4	20% Horsetail powder G5	20mg Horsetail extract G6
Initial weight (g)	115.55 \pm 3.17 ^a	110.41 \pm 2.50 ^a	113.14 \pm 3.45 ^a	112.33 \pm 2.99 ^a	110.22 \pm 3.11 ^a	110.34 \pm 3.14 ^a
Final Weight (g)	200.47 \pm 10.11 ^a	165.71 \pm 15.6 ^{b**}	189.71 \pm 10.9 ^a	200.3 \pm 13.80 ^a	205.5 \pm 12.00 ^a	203.44 \pm 15.8 ^a
Weight Gain (g)	88.92 \pm 11.33 ^a	53.30 \pm 7.71 ^{b**}	79.57 \pm 8.17 ^a	85.08 \pm 10.22 ^a	90.92 \pm 11.11 ^a	93.77 \pm 11.21 ^a
Food Intake (g/d)	16.65 \pm 2.11 ^a	13.20 \pm 2.17 ^a	15.90 \pm 2.11 ^a	16.35 \pm 2.91 ^a	16.55 \pm 2.18 ^a	16.75 \pm 2.81 ^a
FER	0.093 \pm 0.001 ^a	0.051 \pm 0.002 ^{b**}	0.084 \pm 0.003 ^a	0.092 \pm 0.001 ^a	0.091 \pm 0.001 ^a	0.094 \pm 0.003 ^a
PER	0.46 \pm 0.03 ^a	0.25 \pm 0.01 ^{b**}	0.42 \pm 0.03 ^a	0.46 \pm 0.02 ^a	0.45 \pm 0.04 ^a	0.47 \pm 0.03 ^a

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each row having different superscript (a, b, c) denote significant difference.

The effect of tartrazine and horsetail (powder and extract) on blood hemoglobin (HB) and packed cell volume (PCV) of the experimental rats groups.

Results in Table (2) showed that administration of TZ decreased the value of hemoglobin (HB) and packed cell volume (PCV) in compared to control negative group. The HB and PCV values of positive control group was 6.19 gm/dl and 29.41%, meanwhile it was 14.08 gm/dl and 49.09%, respectively for negative control group. The treatment with horsetail (powder and extract) reversed the effect of TZ, as there was a significant increase in levels of HB and PCV in comparing to control positive group. It could be noticed that there were no significant changes between horsetail (powder and extract) groups and negative control group. The results of HB and PCV support the reports of

Daffallah et al. (2015); Aboel-Zahah et al. (1997); Sharma et al. (2009) and Abd-Elhakim et al. (2018 A) who revealed that the mean red blood cells (RBC), HB, PCV, and platelet count values were significantly decreased following treatment with tartrazine compared to the control group values. **Elekima and Christian (2019)** stated that administration of high doses far above the ADI of tartrazine induced decrease HB however, chronic treatment at ADI doses showed no significant difference after 30, 60, and 90 days. It is likely that the absorption of TZ and its metabolites in the blood plasma will adversely affect HB function and can even lead to impairment of its activity, as TZ can bind strongly to HB and induce significant conformational changes its transportation and metabolism in the human body can pose potential biological toxicity risk (**Basu and Kumar, 2016**). The interaction between HB and TZ possibly due to hydrogen bonding, hydrophobic and hydrophilic interactions (**Mandal and Ganguly, 2009** and **Lu et al., 2007**). However, **Zokian and Mohamad (2010)** found that *Equisetum arvense* L. crude extracts (water and ethanolic) of (10, 50 mg/ml), significantly, increased the amount of hemoglobin *in vivo*, whereas higher concentration of (100mg/ml) decrease it.

Table (2): Mean values \pm SD of blood hemoglobin (HB) and packed cell volume (PCV) of the experimental rats groups.

Groups Variables	Normal control G1	Positive control G2	10%Horset ail powder G3	10mgHorset ail extract G4	20%Horset ail powder G5	20mgHorset ail extract G6
HB (gm/dl)	14.08 \pm 1.18 ^a	6.19 \pm 0.39 ^{b**}	11.04 \pm 1.4 ^a	10.15 \pm 1.98 ^a	12.08 \pm 2.01 ^a	11.44 \pm 1.82 ^a
PCV %	40.09 \pm 3.82 ^a	29.41 \pm 3.55 ^{b*}	35.79 \pm 3.47 ^{b*}	37.14 \pm 4.01 ^a b	36.11 \pm 4.11 ^a	38.81 \pm 3.17 ^a

Sig nificant with control (-ve) group * P<0.05 ** P<0.01 ***

P<0.001

Mean values in each raw having different superscript (a, b, c) denote significant difference.

Effect of tartrazine and horsetail (powder and extract) on some liver and kidney functions of the experimental rats groups:

Data in Table (3) showed that treatment with tartrazine resulted in a significant ($p < 0.05$) increase in the activity of plasma aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) compared to negative control group. Also, it showed a significant ($p < 0.05$) increase in plasma uric acid and creatinine levels in tartrazine-treated animals. Meanwhile, treatment with horsetail powder or extract nearly restored the levels of AST, ALT, ALP, uric acid and creatinine when compared to the TZ treated group (Table 3). It could be noticed that horsetail extract fulfilled this role with slight more competence than horsetail powder. These results are parallel to **Aboel-Zahab *et al.* (1997); Mekkawy *et al.* (1998) and Sharma *et al.* (2005)** who indicated that TZ administration showed significant increases in serum AST, ALT, and alkaline phosphates activities. **Mekkawy *et al.* (1998); Himri *et al.* (2011); El-Wahab and Moram (2015) and Saxena and Sharma (2015)** attributed these results to hepatocellular damage caused by the toxic effects of these synthetic dyes which indicated by vacuolation, swelling, necrosis and pyknosis of the liver cells, This liver damage would releases greater than normal levels of intracellular enzymes into the blood. In the same concern, **Amin *et al.* (2010)** revealed that low and high doses of TZ induced significant increases in serum ALT, AST and ALP activities, also increased urea and serum creatinine levels in comparison to control group. Furthermore, **Helal *et al.* (2000)** found that oral administration of synthetic or natural colorants induced a marked increase in the serum AST, ALT, urea and creatinine levels of all treated groups after 30 days of treatment. **Ashour and Abdelaziz (2009)** observed significant elevations in serum creatinine and urea levels of rats dosed with organic azo dye (fast green) orally for 35 days. **Tawfek *et al.*, (2015)** found a significant increase in serum creatinine and urea in rats following the consumption of different types of feed additives including tartrazine, sunset

yellow and sodium benzoate. **Mehedi et al. (2013)** mentioned that tartrazine application induced significant elevations in urea and creatinine levels that is associated with impaired renal function and the inability of the kidney to filter body fluids. **Khayyat et al. (2017)** reported that the consumption of azo dyes caused a marked increase in the levels of AST, ALT, urea, uric acid and creatinine in rats. **Arefin et al. (2017)** revealed that TZ significantly increased serum creatinine and bilirubin levels. However, the pretreatment effect of *E. arvense* extract (25, 50, and 75 mg/kg) was remarkably protected against both liver and renal injury caused by CCl₄. Where *E. arvense* extract significantly decreased serum ALT, AST, and ALP, creatinine, uric acid, and urea, as well as improve the serum protein and albumin levels (**Ragheb and Alamri, 2020**). **Katikova et al. (2002)** investigated the hepatoprotection activity of *E. Arvense* herbs extract in a model of acute hepatitis produced by tetrachloromethane. The results offered that the extract protected the membrane through antioxidant action. This was displayed through lowered liver enzymes, total bilirubin, and lipid peroxidation products.

Table (3): Effect of tartrazine and horsetail (powder and extract) on some liver and kidney functions of the experimental rats groups

Groups Variables	Normal control G1	Positive control G2	10%Horset ail powder G3	10mgHorse tail extract G4	20%Horset ail powder G5	20mgHorset ail extract G6
AST (μ/ml)	55.17± 5.81 ^b	72.39± 9.61 ^{a**}	49.37± 6.01 ^b	51.14± 8.10 ^b	48.21± 6.15 ^b	40.21± 4.13 ^b
ALT (μ/ml)	12.35± 1.12 ^b	28.55± 3.35 ^{a**}	15.71± 1.81 ^b	16.28± 2.01 ^b	18.13± 3.51 ^b	14.11± 3.65 ^b
ALP (μ/ml)	31.17± 5.66 ^b	50.38± 5.81 ^{a**}	37.80± 4.11 ^b	38.73± 4.37 ^b	38.34± 5.01 ^b	36.11± 3.11 ^b
Creatinine (mg/dl)	0.77± 0.01 ^b	1.95± 0.11 ^{a**}	0.99± 0.02 ^b	0.88± 0.12 ^b	0.75± 0.13 ^b	0.70± 0.15 ^b
Uric acid (mg/dl)	1.83± 0.26 ^c	4.41± 1.01 ^{a***}	2.11± 0.81 ^{b*}	2.41± 0.77 ^{b*}	2.17± 0.67 ^{b*}	1.74± 0.74 ^c

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001

Mean values± SD in each column having different superscript (a, b, c) denote significant difference.

AST: aspartate transferase

ALT: alanine aminotransferase

ALP: alkaline phosphatase

Oh et al. (2004) showed that the methanolic extract of *E. arvense* produced a marked protective action against tacrine-prompted cytotoxicity in the Hep G2 cell line. Values of serum AST, ALT, ALP, uric acid and creatinine decreased gradually with increasing the level of horsetail as compared to the positive control group treated with prednisone acetate (**Arafa, 2016**). Administration of *E. arvense* to streptozitocin-induced diabetic rats for month lowers the level of serum glucose, urinary creatinine and microalbuminuria (**Soleimani et al., 2007**).

Effect of tartrazine and horsetail (powder and extract) on plasma oxidative/antioxidant biomarkers

The activity of antioxidants enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) and oxidative stress biomarker nitric oxide (NO) revealed significant changes among different groups as shown in Table (4). The TZ group showed an obvious significant decrease in the activity of CAT ($105.55 \pm 10.14 \mu / l$ plasma), SOD ($20.25 \pm 3.47 \mu / l$ plasma) and GST ($77.85 \pm 8.40 \mu / l$ plasma) when compared with the control group (385.21 ± 55.14 , 70.13 ± 5.22 and $288.31 \pm 33.27 \mu / l$ plasma, respectively), while treatment with horsetail powder or extract nearly restored the levels of CAT, SOD and GST when compared to the TZ treated group (Table 4). A significant increase in the concentration of plasma NO level was seen in the TZ group ($13.99 \pm 1.44 \mu mol / l$ plasma) compared to the control group ($2.17 \pm 0.33 \mu mol / l$ plasma), as well as other treated groups. On the other hand, this increase was mitigated in the horsetail groups, the 10% and 20% horsetail powder groups (4.33 ± 1.11 and $3.11 \pm 1.05 \mu mol / l$ plasma, respectively) and the 10mg and 20mg horsetail extract groups (3.22 ± 1.03 and $2.01 \pm 1.21 \mu mol / l$ plasma, respectively), showing that horsetail extract fulfilled this role with slightly more competence than horsetail powder.

Oxidative stress is referred to a reactive oxygen species (ROS)/antioxidant imbalance. It occurs when the overall level of ROS exceeds the potential of the antioxidants. Thus, oxidative stress may occur because of accelerated ROS production, a drop of the antioxidant mechanisms, or both (France's *et al.*, 2013). In the present study, elevated levels of NO clearly indicates oxidative stress occurrence in the tartrazine-treated rats. Where tartrazine metabolized inside the body into aromatic amines by intestinal microflora. These formed amines can generate ROS as part of their metabolism by the interaction of the active amino groups with nitrite or nitrate containing foods (Moutinho *et al.*, 2007). NO is considered as important source of free radicals that might contribute to alterations in energy metabolism. Peresleni *et al.* (1996) demonstrated that oxidative stress to epithelial cells increases NO syntheses which results in elevated NO release, nitrite production and decreased cell viability. Also, the present results are in accordance with Khayyat *et al.* (2017) who reported that TZ consumption caused a marked increase in the levels of MDA and NO and a decreased level of total antioxidants in the serum of rats dosed with tartrazine compared to control group. Tufarelli *et al.* (2021) found that the concentrations of serum total superoxide dismutase (TSOD) and total antioxidant capacity (TAC) increased however, MDA decreased by adding horsetail to hen diet compared to control diet.

Table (4): Effect of tartrazine and horsetail (powder and extract) on plasma oxidative/antioxidant biomarkers

Groups Variables	Normal control G1	Positive control G2	10% Horsetail powder G3	10mg Horsetail extract G4	20%Horset ail powder G5	20mgHorset ail extract G6
GST (μ /l)	288.31 \pm 33.27 ^a	77.85 \pm 8.40 ^{c***}	188.35 \pm 22.17 ^{b*}	211.31 \pm 23.81 ^{b*}	240.21 \pm 23.71 ^a	278.15 \pm 31.71 ^a
SOD (μ /l)	70.13 \pm 5.22 ^a	20.25 \pm 3.47 ^{b***}	63.14 \pm 7.16 ^a	68.33 \pm 6.35 ^a	71.31 \pm 9.23 ^a	73.14 \pm 7.81 ^a
Catalase (CAT) (μ /l)	385.21 \pm 55.14 ^a	105.55 \pm 10.14 ^{c***}	230.77 \pm 32.11 ^{ab}	291.61 \pm 31.61 ^a	277.11 \pm 30.91 ^a	384.11 \pm 39.11 ^a
NO (μ mol/l)	2.17 \pm 0.33 ^b	13.99 \pm 1.44 ^{a***}	4.33 \pm 1.11 ^b	3.22 \pm 1.03 ^b	3.11 \pm 1.05 ^b	2.01 \pm 1.21 ^b

Significant with control (-ve) group * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Mean values \pm SD in each column having different superscript (a, b, c) denote significant difference.

GST: glutathione-S-transferase

SOD: superoxide dismutase

NO: enzymes and nitric oxide

Effect of tartrazine and horsetail (powder and extract) on oxidative/antioxidant biomarkers of liver homogenate tissue:

The activity of antioxidant of liver homogenate tissue SOD, glutathione peroxidase (GPX), GST, and oxidative stress biomarker malondialdehyde (MDA) revealed significant changes among different groups as shown in Table (5). The TZ group showed an obvious significant decrease in the activity of GPX ($25.14 \pm 3.19 \mu$ /mg tissue), SOD ($35.81 \pm 3.81 \mu$ /mg tissue) and GST ($1.51 \pm 0.19 \mu$ /mg tissue) when compared with the control group (130.33 ± 17.13 , 155.81 ± 21.17 and $5.6 \pm 0.66 \mu$ /mg tissue, respectively), while treatment with horsetail powder or extract nearly restored the levels of GPX, SOD and GST when compared to the TZ treated group (Table 5). Significant increase in the concentration of MDA in liver tissue homogenate was seen in the TZ group (19.34 ± 3.14 mmol/g tissue) compared to the control group (9.45 ± 1.98 mmol/g tissue), as well as other treated groups. On the other hand, this increase was mitigated in the horsetail groups, the 10% and 20% horsetail powder groups (10.14 ± 2.61 and 10.33 ± 1.69 mmol/g tissue, respectively) and the 10mg and 20mg horsetail extract groups (9.11 ± 2.16 and 8.22 ± 1.91 mmol/g tissue, respectively), showing that horsetail extract fulfilled this role with slightly more competence than horsetail powder. These results are in agreement with **Omar, (2008)** who reported that oral administration of TZ for one month evoked a significant decrease in the activity of CAT and SOD and concentration of GSH as well as a significant increase in the concentration of MDA, a byproduct of lipid peroxidation, considered as an indicator of oxidative stress. Also, rats consumed low and high doses of TZ showed significant decreases in liver catalase, SOD and GSH activities, however it showed significant

increase in liver MDA in comparison to control group (**Amin *et al.*, 2010**). This may be attributed to the presence of the azo group that binds to aromatic rings in the molecular structure of TZ. Oral consumption of TZ induces reactive oxygen species (ROS) due to the formation of aromatic amines (nitro azo dye) through the action of azo reductase enzyme present in the intestinal microflora (**Bansal, 2005** and **Umbuzeiro *et al.*, 2005**). As a result of ROS formation of the antioxidant defense mechanism of the cells including catalase, SOD, and GSH began to consumed to prevent the cell death by these toxic radicals so their levels in the tissue homogenate were decreased specially at higher doses when the need for them was increased, on the other hand MDA level was increased as a product of lipid peroxidation occurred by the ROS action on lipids of cellular membrane (**Bansal, 2005**). Similarly, **Gao *et al.* (2011)** and **Mohamed *et al.* (2015)** found that the administration of TZ for 30 days resulted in a decline in the activities of CAT, glutathione peroxidase, and SOD while there was a rise in the level of MDA. TZ resulted in significantly increase of both MDA and total protein concentrations also, it resulted in statistically significant decrease of reduced glutathione (GSH), SOD, CAT and GPx concentrations (**El Desoky *et al.*, 2017**). **Eman *et al.* (2018)**, added that oral tartrazine at a daily dose of 50 mg/kg for 30 days showed significant decrease of GPx and significant increase of MDA levels in the cerebellar tissue.

Table (5): Effect of tartrazine and horsetail (powder and extract) on oxidative/antioxidant biomarkers of liver homogenate tissue

Groups Variables	Normal control G1	Positive control G2	10%Horsetail powder G3	10mgHorsetail extract G4	20%Horsetail powder G5	20mgHorsetail extract G6
SOD (μ /mg)	155.81 \pm 21.17 ^a	35.81 \pm 3.81 ^{b***}	110.15 \pm 11.15 ^a	131.25 \pm 22.61 ^a	118.82 \pm 17.34 ^a	143.32 \pm 25.16 ^a
GPX (μ /mg)	130.33 \pm 17.13 ^a	25.14 \pm 3.19 ^{c***}	89.59 \pm 7.95 ^{b*}	118.41 \pm 11.18 ^a	114.38 \pm 13.21 ^a	120.33 \pm 21.35 ^a
GST (μ /mg)	5.6 \pm 0.66 ^a	1.51 \pm 0.19 ^{c***}	2.99 \pm 0.88 ^{b*}	3.22 \pm 0.97 ^a	3.29 \pm 0.77 ^a	4.11 \pm 0.98 ^a
MDA (nmol/g)	9.45 \pm 1.98 ^b	19.34 \pm 3.14 ^{a***}	10.14 \pm 2.61 ^b	9.11 \pm 2.16 ^b	10.33 \pm 1.69 ^b	8.22 \pm 1.91 ^b

Significant with control (-ve) group * $P < 0.05$ ** $P < 0.01$ ***
 $P < 0.001$

Mean values in each column having different superscript (a, b, c) denote significant difference.

Albasher et al. (2020) revealed that oral tartrazine within the ADI (2.5 and 5 mg/kg daily) provoked significant increase of MDA in different brain regions. Also, **Hosieny et al. (2021)** showed that in tartrazine-treated group there was highly significant reduction in the mean value of GPx activity and highly significant elevation in the mean value of MDA level when compared with control groups. On the other hand, **Ragheb and Alamri (2020)** found that *E. arvense* extract decline serum level of MDA induced by CCl_4 injection. This effect was further explained by *E. arvense* phytochemical antioxidant constituents, which possesses a potent radical scavenging ability (**Khan et al., 2013; Park and Jeon, 2008 and Rehman et al., 2018**).

Conclusion:

The results of this study elucidated the promising protective role of horsetail (powder and extract) on hematological and biochemical profiles in hepatic and renal function, the antioxidant and oxidative stress biomarkers in the plasma and liver homogenate tissues parameters against TZ-toxicity in rats. The mechanism behind *E. arvense* action could be explained by its antioxidant and free radicals scavenging efficacy. Meanwhile, the consumption of foods containing food colorant tartrazine should be restricted as far as possible. However, further investigations should be carried out on different products to achieve full protection.

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التأثير الوقائي لنبات ذيل الحصان (*Equisetum arvense* L.) ضد سمية صبغة الأزو الغذائية التارترازين علي بعض المعايير البيوكيميائية ومضادات الأكسدة لدى ذكور الفئران: دور الإجهاد التأكسدي

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الملخص

هناك اهتمام متزايد في الوقت الحاضر باستخدام النباتات الطبية، هذا و يعتبر لنبات ذيل الحصان (*Equisetum arvense* L.) من العائلة Equisetaceae استخدامات عديدة في الطب التقليدي وله العديد من التأثيرات الدوائية التي يرتبط معظمها بتأثيراته المضادة للأكسدة. ومن جانب آخر تعتبر التارترازين (TZ) مادة صناعية من صبغات الأزو العضوية التي تستخدم على نطاق واسع كمادة ملونة مضافة للأغذية والأدوية ولمستحضرات التجميل و من الممكن أن تؤدي إلى الإجهاد التأكسدي الذي يؤدي بالتالي اضطرابات أيضية مثل تسمم الكبد والكلية. ولهذا تم تصميم هذه الدراسة لتقييم الخصائص الوقائية لنبات ذيل الحصان ضد الإجهاد التأكسدي المحدث بواسطة TZ في الفئران حيث تم تقسيم اثنين وأربعين فأراً بشكل عشوائي إلى ست مجموعات (٧ فئران لكل مجموعة). تم الاحتفاظ بالمجموعة الأولى (G1) بدون أى معالجة وسميت بالمجموعة الضابطة السالبة، بينما أعطت المجموعات الخمس الأخرى جرعة من التارترازين فمويًا ٣٠٠ مجم/كجم من وزن الجسم/يوم. وتم الاحتفاظ بمجموعة واحدة كمجموعة ضابطة موجبة (G2)، في حين عولجت باقي مجموعات الـ TZ الأخرى بمسحوق ومستخلص ذيل الحصان على النحو التالي: المجموعة الثالثة (G3) بمسحوق ذيل الحصان ١٠٪/كجم وجبة/يوم، المجموعة الرابعة (G4): ١٠ مجم مستخلص ذيل الحصان/كجم من وزن الفئران، المجموعة الخامسة (G5) ٢٠٪ مسحوق ذيل الحصان/كجم وجبة/يوم والمجموعة السادسة (G6): ٢٠ مجم مستخلص ذيل الحصان/كجم من وزن الفئران. ولقد أظهرت النتائج أن المعالجة بذيل الحصان سواء المسحوق أو المستخلص في جميع الجرعات أدى إلى تحسن معنوي، حيث أدى إلى زيادة أوزان أجسام الفئران، بالإضافة إلى ارتفاعا معنوا لكلا من HB، PCV، وظائف الكبد (ALT، AST و ALP) ووظائف الكلى (الكرياتينين وحمض البوليك) بالمقارنة بالمجموعة الضابطة الموجبة (TZ). وارتبط ذلك بارتفاع كبير للمؤشرات الحيوية لمضادات الأكسدة في البلازما GST و SOD و CAT وانخفاض العلامات الحيوية للإجهاد التأكسدي NO، وكذلك للمؤشرات الحيوية لمضادات الأكسدة في أنسجة الكبد، حيث أدت المعالجة بذيل الحصان إلى زيادة نسب كلا من GST و SOD و GPX معنويًا وانخفض مستوى الـ MDA معنويًا بالمقارنة بالمجموعة الضابطة الموجبة (TZ). الخلاصة: يمكن الاستنتاج بأن المعالجة بذيل الحصان سواء المسحوق أو المستخلص أظهرت دوراً وقائياً واضحاً ضد التأثير السلبى للتارترازين وغيرت العلامات البيوكيميائية لأعضاء الحيوية مثل الكبد والكلية وارتبط ذلك بتقليل الإجهاد التأكسدي وذلك من خلال تأثيره المضاد للأكسدة.

الكلمات المفتاحية: التارترازين، ذيل الحصان (*Equisetum arvense*)، الهيموجلوبين، مضادات الأكسدة، الإجهاد التأكسدي، وظائف الكلى والكبد.