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Study of the Effect of Sudan II and Treatment water Coupled with Fe^{2+} in Modulating Hematological and Biochemical Changes in Rabbits'

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Abstract

Throughout the years, azo compounds including Sudan dyes have been widely used in industry as colorants in food, cosmetics, waxes, solvents, textiles, plastics and printing inks. Sudan dyes I, II, III, IV, and their degradation products have been regarded to hurt human health. They cause cancer due to their teratogenicity, genotoxicity and carcinogenicity effects. In the present work, the efficiency of ferrous sulfate with low cost and the first choice in the treatment of printing, dyeing and electroplating wastewater to reduce the toxicity of Sudan II-induced oxidative damage to hematological parameters, liver and kidney functions in adult female and male rabbits was investigated. Animals which received Sudan II were treated with ferrous sulfate as the sole source of potent liquid for 14 days at the dose of 111mg/kg B.wt before being sacrificed. Hematological parameters (total leukocytes (WRBs), erythrocytes (RBCs) count hemoglobin content (Hb) and hematocrit percentage (Ht%), liver and kidney functions were done to check the efficiency of ferrous sulfate as Sudan II detoxification. The obtained data clearly showed that Sudan II toxicity led to a significant reduction in (WBCs, RBCs, Hb, and Ht) of the hematological parameters. Sudan II led to an elevation in the activity of liver function enzymes (transaminase; ALT and AST), a decrease in Alkaline phosphatase ALP and an increase in kidney functions (urea and creatinine). The detoxification properties of ferrous sulfate were obvious thanks to good flocculation and decolorization, utilized to separate heavy metal ions, oils, phosphorus and forpurification, and wastewater. Finally, the present study proved that Sudan II treated with ferrous sulfate can keep hematological parameters, liver and kidney functions near the physiological normal state.

Keywords: Ferrous sulfate, Sudan dyes, liver and kidney functions and rabbits.

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Introduction

Synthetic or natural food additives may be used as flavoring or coloring tools (Sayed et al., 2012). Moreover, Amin et al. (2010) explained that colorants provide an aesthetic appearance to foods preferred by consumers. In this context, most of Azo dyes which are artificial colors and do not occur naturally contained only one azo group, but some contained two (di-azo), three (tri-azo) or more. Moreover, Kusic (2011) classified Azo dyes according to their color, origin, chemical structure and the type of material. Humans' exposure to azo dyes occurs through inhalation, ingestion and skin contact. Critic Shore (1996) warned that this may be quite harmful due to the possible production of carcinogenic amines.

Critics Rathod and Thakre (2013) elaborated that "Sudan dyes are azo compounds, which constitute one of the largest classes of industrially synthesized organic compounds, potent in drug and cosmetics." Furthermore, critics Yan et al., (2011) found Sudan azo dyes in different foods as chili powders, eggs, Worcestershire sauce, garlic curry sauce, palm oils and also in other food products. According to Calbiani et al, (2004) the main reason for their widespread usage is their colorfastness, low price, and their intense red color.

More importantly, Azo dyes are constant in the pH of foods and heat, and they do not faint when exposed to light or oxygen. Moreover, they are not soluble in oil or fat, and it can be observed that when the azo dyes connected with a fat-soluble molecule, the oils can be colored.

Stahlmann (2006) asserted that the azo linkage may be reduced, and it was demonstrated that this reaction was done by an enzyme namely azo-reductase. It is a non-specific enzyme that may be found in various micro-organisms existing in various human organs. Azo dyes have not been given the authorization to be

utilized as food additives due to their potential carcinogenicity. The International Agency for Research on Cancer (IARC) has classified Sudan from I to IV as class 3 carcinogen (IUPAC, 1997).

Sudan II (Cas No. 3118-97-6) {1-[(2, 4-dimethylphenyl) azo]-2-naphthalenol} or [1-(2, 4-xylylazo)-2-naphthol], also known as Solvent Orange 7, is a hydrophobic fat-staining dye. Sudan dyes are maybe lipid-soluble dyes namely lysochromes which are di-azo dyes. Sudan II is used for confirmed solvents, and may also be utilized to stain some protein bound lipids in paraffin. Cameron (1987) studied the cellular areas where the dye is sequestered, Sudan II has been utilized to estimate how toxins reacted with membranes.

The present research was designed to estimate the role of Sudan treated with Ferrous sulfate against Sudan II which induced disorders in hematological and biochemical parameters in liver and kidney of female and male rabbits.

Material and Methods

Experimental animals

The experiment was performed over 2 weeks on 36 rabbits (18 females and 18 males) with the average wt of (90±10g), kept under optimal ventilation, illumination, temperature and relative humidity conditions. It is worthy to note that all animal treatments were in accordance with "the Ethics Committee of the National Research Centre" and "the Guide for the Care and Use of Laboratory Animals"(Derrell et al., 1997). Figure 1 shows the structure of Sudan II.

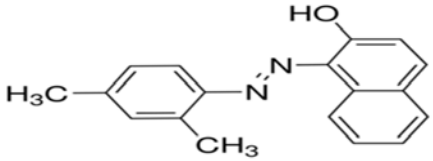

Fig. 1. Structure of Sudan II

Photochemical studies

- ***Instrumentation:*** *2910HITACHI spectrophotometer*, which has been classified as class A of EN61326 was used. The program had the following measurement modes:
  a) Wavelength scan 200-800 nm
  b) Time scan
  c) Photometry

Experiments

The samples of photodegradation behaved in sunlight for degeneration, 0.05 molar solution Sudan II in 25 mL of dye solution was used where Sudan II dissolved in ethanol and water Mixture (1:5) concentration was added and irradiated with sun light at different times, and 0.2% of ferrous sulfate was used. All the experiments were carried out at room temperature (30 ± 0.1□C).

- *Preparation and methods:*

10 ml of aqueous solution of Sudan II was added, then 1 ml of ferrous sulfate was added to conical flask of 25 complete to 25 ml. The above step was repeated, but 2,3,4,5 ml of ferrous sulfate was added. The solutions were irradiated with sun light at different times.
2 Spectrophotometery was used to analyze each solution after all additions.
3- Spectrophotometery was used to analyze each solution before the irradiation of sun light (Rashed and El-Amin, 2007).

Animal groups

The animals were randomly allocated to 3 treated groups of twelve rabbits (6 females and 6 males) each: Group 1(control): Animals were supplied with water ad-libitum. Group 2 (Sudan group): Animals were administered with Sudan II beverage as a sole source of potable liquid for 2 weeks at the dose of 333 mg/kg B. wt. Group 3 (Sudan treated with ferrous Sulphate): Rabbits received Sudan as in Group 2 and treated with ferrous Sulphate .

At the end of biological experiential, blood was collected then centrifuged, and the blood serum was kept on frozen till examined. A portion of blood was collected using an anticoagulant for hematologic parameters.

Red (RBCs) and white (WBCs) blood cell count, hemoglobin content (Hb), hematocrit (Ht), were measured as described by Dacie and Lewis (1993).

Serum biochemical parameters

Liver functions were determined according to Schumann and klauke (2003). Alkaline phosphatase was estimated according to Schlebusch et al., (1974). Serum urea and creatinine were assessed based on Tobacco et al., (1979) and Bartels et al. (1971); respectively.

Statistical Analysis

Mean values between control and treated group was analyzed statistically by the method in the paradigm of Snedecor and Cochran (1980).

Results

Effect of time irradiation for (Sudan II)

In the present research, the methods were used for the removal of Sudan II, and the mechanisms were found to be very beneficial and low-cost for the best elimination of dye and comparison among elimination dyes by ferrous sulfate. The results in (Fig.2) showed that the photodecolorization percent of Sudan II became considerably greater when the time of irradiation increased. From the results, the more photogenerated electron (e−)/hole, the more (h VB) pairs were generated (Assy et al., 2013).

Effect of ferrous sulfate:

In this reaction, there were two effects on the dye structure:

1. Fe^{2+} bonded to N and O atoms present in the dye structure for the adsorption of dyes
2. $HO^{\bullet}$ radical lead was formed (Sawsan, 2015).

$$Fe^{2+} + H_2O + h^{+VB} \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-}$$

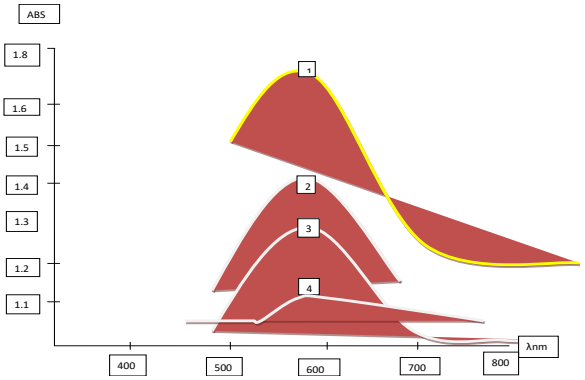

Fig. 2: Effect of time irradiation on Sudan II treated with Fe (II) ions where curve 1 shows Sudan before irradiation , curve 2 shows Sudan 30 min after the irradiation - curve 3 shows Sudan 60 min after the irradiation - curve 4 shows Sudan 120 min after the irradiation.

From the present work, ferrous sulphate has been used as trial to remove the toxicity of Sudan at the concentration of (333mg/kg B.wt) of drinking water. Table 1 introduces the body weight of rabbits in all groups. It was noticed that there were no significant variations in body weight among control and -treated groups. Sudan II induced a significantly more decreased ($p<0.0.05$) body weight than the control group. Besides, there was no considerable variation among females and males in all groups.

Table 2 shows that there was no considerable variation in blood parameters among healthy control and Sudan II -treated with ferrous sulphate groups. Sudan II led to a remarkable decrease in WBCs, RBCs, Hb concentrate ($p<0.0.05$) and Ht% than the control group. There was no significant difference between females and males in all groups. In the treatment groups, the blood parameters were more improved than the irradiated group ($p< 0.05$).

Table 1. Effect of ferrous sulphate administration for 14 days on body weight of female and male rabbits treated with Sudan II

Groups		1st day	7th day	14th day
Control	female	1.35±0.05	1.66±0.11	1.71±0.09
	male	1.30±0.06	1.58±0.06	1.66±0.06
Sudan II(SII)	female	1.28^{ab}±0.04	1.07^{ab}±0.04	1.95^{ab}±0.07
	male	1.16^{ab}±0.03	1.12^{ab}±0.04	1.05^{ab}±0.10
(SII)treated with **Fe^{+2}**	female	1.23^{b}±0.05	1.25^{b}±0.04	1.41^{b}±0.03
	male	1.28^{b}±0.04	1.33^{b}±0.05	1.36^{b}±0.06

Values are expressed as means ± S.E for six rabbits in each group. Data are significant at $p<0.05$.
a: significantly different compared to control.
b: significantly different compared to Sudan II group

Table 2. Effect of ferrous sulphate administration for 14 days on blood parameters in female and male rabbits treated with Sudan II

Groups	sex	WBCs (10^3/mm^3)	RBCs (10^6/mm^3)	Hb (g/dl)	Ht (%)
Control	female	13.71±0.33	3.80±0.15	21.55±0.10	44.50±0.15
	male	13.50±0.32	4.76±0.09	23.10±0.22	46.65±0.09
Sudan	female	8.16^{ab}±0.14	2.66^{ab}±0.11	15.44^{ab}±0.15	25.65^{ab}±0.06
	male	7.95^{ab}±0.21	2.95^{ab}±0.16	13.38^{ab}±0.22	24.71^{ab}±0.13
(SII)treated with **Fe^{+2}**	female	12.10^{b}±0.32	4.05^{b}±0.17	20.10^{b}±0.34	42.95^{b}±0.19
	male	11.90^{b}±0.30	3.90^{b}±0.13	20.36^{b}±0.43	44.65^{b}±0.25

Legend as in Table 1

As shown in Table 3, there were no considerable variations among females and males in all groups. There were no significant variations in liver functions between control and Sudan II -treated with ferrous sulphate group. γ -Sudan II showed the highest AST and ALT, meanwhile ALP was lower in comparison with the control rabbits. Treatment with ferrous sulphate showed a

significant decrease ($p< 0.05$) of serum AST and ALT in addition to an increase in ALP than the control group.

Table 3. Influence of ferrous sulphatemanagement for 14 days on the activities of liver functions in female and male rabbits treated with Sudan II

Groups	sex	AST(U/L)	ALT(U/L)	ALP(U/L)
Control	female	43.98±0.13	70.85±0.17	263.93±0.14
	male	44.88±0.21	69.05±0.55	284.03±0.06
Sudan	female	65.16^{ab}±1.3	82.35^{ab}±0.19	138.61^{ab}±0.16
	male	61.95^{ab}±1.1	82.95^{ab}±0.22	188.29^{ab}±0.15
(SII)treated with $\mathbf{Fe^{+2}}$	female	44.35^{b}±0.21	68.18^{b}±0.14	260.31^{b}±0.31
	male	44.18^{b}±0.19	67.15^{b}±0.33	280.28^{b}±0.04

Legend as in Table 1

Results in Table 4 evinced that there were no major variations in serum levels of kidney functions of ferrous sulphate group than the health control. Sudan II led to be considerably higher ($p < 0.05$) in serum levels of kidney functions than the health control. Moreover, ferrous sulphate groups showed to be significantly lower ($p < 0 .05$) in serum levels of kidney functions than Sudan II group.

Table 4. Influence of ferrous sulphate administration for 14 days on urea and creatinine in female and male rabbits treated with Sudan II

Groups	Sex	Urea	Creatinin
Control	female	22.85±0.07	0.84±0.02
	Male	21.96±0.02	0.86±0.01
Sudan	Female	28.65^{ab}±0.19	1.30^{ab}±0.06
	male	38.28^{ab}±0.23	1.41^{ab}±0.12
(SII)treated with $\mathbf{Fe^{+2}}$	female	23.51^{b}±0.20	0.76^{b}±0.01
	male	22.73^{b}±0.24	0.78^{b}±0.02

Legend as in Table 1

Discussion

In a study done by Atkins and Lowe (1979), the removal of Sudan II from wastewater by using both irradiated source such as sunlight and ferrous sulphate was the best recycle, and reutilized the limited water source of the earth especially in textile and dyeing industries, where large quantities of water were used in dyeing and washing /rinsing of the fabrics. Ferrous sulphate could increase the removal of dye and completely decolorize. These techniques used by Adil Al-Hemiri et al., (2007) were found to be very easy and inexpensive**.**

Table 1 shows that Sudan dye causes significant reduction in body weight in the 7^{th} and 14^{th} days; that is why Sudan dye II has been considered harmful to human health. Scholar Alim (2016) asserted that Teratogenisity, genotoxisity and carcinogenesity would lead to cancer. The results of this study matched with the findings of Sharma et al. (2009), who showed that there was a highly significant decrease in the body weight of experimental animals when fed with Tartrazine in higher doses. While Helal et al. (2000) found lower body weights in very young rats fed with many different other synthetic food colorants including SY indicating differential effects on body weight gains between young and old experimental animals. Gautam *et al* (2010) studied the toxic impact of Tartrazine, another food coloring azo dye, on Swiss albino mice, and also found an increase in body weight gain in both experimental groups for low dose (200 mg/kg BW) and high dose (400 mg/kg BW) groups.

Hematological estimates were worthy tools for evaluating the damaging reasons of the confirmed materials. Lowering RBC has been generally shown to cause anemia. The findings about the hematological parameters in the present study showed a significant decrease in WBCs, RBCs counts, Hb concentration and Ht % in animals that were treated with Sudan II compared to the control group. These hematological changes indicated that anemia might occur as a result of the effect of Sudan dye. This matched with the ideas of Awad et al., (2017) who cited that hair dye caused chocolate brown urine as an indicative of haemoglobinurina resulting from haemolysis. Moreover, Hopkins and Manoharan (1985) found out that hair dyes had toxic effects on bone marrow, and can suppress the reduction of blood cells. In their steps, Chakravarty *et al.* (2005) observed that there was a decrease in hemoglobin and total erythrocyte count at all dose levels of the food dye utilized.

A similar finding was also reported by Mohammed (2014) who cited that there was a marked decrease of red blood cells counts, hemoglobin of male albino rats treated with a various doses of tartrazine for 30 days. In contrast, Ikechukwu (2017) identified a significant increase in haemoglobin (Hb) and red blood cell count (RBC) in animals, which were fed the low dose of amaranth compared to the control group.

Makni *et al* (2001) asserted that cytoplasmic enzymes such as ALT, AST and ALP were employed in the assessment of hepatic disorders, and the elevation in these enzymes activities reflected liver damage and inflammatory hepatocellular disorders. Mittelstaedt et al. (2004) explained that the increase in plasma AST and ALT activities in Sudan group might be referred to the hepatocellular injury that occurred from chemical toxicity and in

these enzyme levels a close connection to cell necrosis and/or greater cell membrane permeability was observed that led to the emptying of the enzymes to the bloodstream, and there was a high level in serum. More significantly, the results of this study coincided with the study of Samar et, al., who detected a significant increase in liver enzymes (GOT and GPT) after the oral administration of Sudan III for 45 days in low and high doses. The results of this study also agreed with the findings of Salih El-Amin (2014), who detected a significant increase in liver enzymes (GOT and GPT) activities in a dose-dependent manner after oral or sub-cutaneous administration of hair dye.

PPD is the main constituent in hair dye and is a derivative of paranitro- aniline. The present study noticed a decrease in alkaline phosphatase (ALP) activity in rabbits treated with Sudan II. This was suggestive of the liver muscles injury. These results were in accordance with a study done by Anugrah (2010). On the contrary, Shinnawy and Elkattan (2013), and Beenam and Shiv (2015) cited that rats treated with a higher dose of tartrazine showed an increase in ALP. Helal (2001) studied the effect of oral administration of a mixture of sodium nitrate and SY given daily for 30 days to rats, and showed a significant increase in the serum ALP level.

The results of this study indicated that the increased levels of kidney functions in animals were due to the fact that they were treated with Sudan II. This study was in agreement with Ashour and Abdelaziz (2009), showed that there was a significant increase in the serum kidney functions of rats which were orally treated with organic azo dye for 35 days. The increase in the blood urea might be caused by hydronephrosis; and congenital cystic and kidney tuberculosis might be due to the deposition of calcium and also hypervitaminosis D. “Plasma creatinine’s increase in renal diseases gave more noticeable significance than those of the other nitrogenous substances”: Helal et al. (2000) corroborated.

Conclusion

Sudan II adversely altered blood parameters, hepatic enzymes and kidney function .This highlighted the importance and risk of azo dye existing in human food.

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