http://dx.doi.org/10.4314/jpb.v9i2.1
Vol. 9 no. 2, pp. 55-61 (September 2012)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Biochemical and histological evaluation of the effect of Sudan IV (a red dye) on renal function

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Received 9th June 2012; Accepted 27th July 2012

Abstract
The effect of a red dye, Sudan IV on kidney function was investigated. Some biochemical parameters such as total protein, creatinine, urea, sodium, potassium, alanine aminotransferase and aspartate aminotransferase levels were determined. Histopathological analyses were also carried out. Consequently, thirty-two albino rats (Wistar strain) were divided into 4 groups of eight rats each. The groups were given 90% rat chow supplemented with 10% palm oil. Sudan IV was administered in the diet to provide levels of 0% (PO), 0.005% (PO/0.005), 0.01% (PO/0.01) and 0.015% (PO/0.015). They were given these diets for six weeks along with water *ad libitum.* Weekly measurements of weights were recorded. Results showed that there were no significant ($P<0.05$) differences in weight gain although there was slight reduction in feed intake. A significant ($P<0.05$) increase in the levels of total protein, creatinine, urea, aspartate aminotransferase and alanine aminotransferase levels suggest possible kidney malfunction. Histopathological analyses reveal damage to renal tissues in rats administered with the dye.

Keywords: Sudan IV; Kidney; Creatinine; Urea; Histopathological; Biochemical parameters

INTRODUCTION

The group of colour additives known as Sudan dyes consists of a number of red colours e.g. Sudan I through IV, Sudan orange G, Sudan red B, and Sudan red 7B. These groups of dyes are basically synthetically produced red azo dyes used in colouring solvents, oils, waxes, petrol, shoe and floor polishes. Sudan I, III and IV have been classified as category 3 carcinogens by the International Agency for Research on cancer (Refat *et al*., 2008). Although the use of Sudan IV as food additive had been banned in many countries because of its possible carcinogenic effect (Graham, 2008), sub lethal levels of this dye are used in some developing countries of the world to enhance the colour of palm oil. Some workers have reported the toxic effect of some azo dyes even at sub-lethal levels (Dees *et al*., 1997, Tsuda *et al*., 2001, Vorhees *et al*., 1983). Dees *et al.* (1997) observed that Red No. 3 could be a significant risk factor in human breast carcinogenesis. Vorhees *et al.* (1983) reported evidence of physical and behavioural toxicity in developing rats given 10% diet containing FD and C Red No. 40 for two weeks.

Palm oil has been used in food preparation for over 5,000 years. Palm oil, obtained from the fruit of the oil palm tree, is the most widely produced edible vegetable oil

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ISSN 0189-8442 © 2012 Faculty of Pharmaceutical Sciences, University of Jos, Jos. Nigeria.

in the world and its nutritional and health attributes have been well documented (Chondrasekharan *et al*., 2000). It is a common cooking ingredient in the tropical belt of Africa, South East Asia and parts of Brazil. Its increasing use in the commercial food industry in other parts of the world is as a result of its lower cost and high oxidative stability of the refined product when used for frying (Cheman *et al*., 1999, Matthaus, 2007).

In some developing countries where legislature banning the use of Sudan IV is not well enforced, a large number of people consume Sudan IV adulterated palm oil diet on daily basis. In addition to the possible carcinogenic effect of Sudan IV dye, it may also exert other toxic effects on vital organs of the body. Chronic kidney disease is a worldwide public health problem and is now recognized as a common condition that is associated with an increased risk of cardiovascular disease. It is a common cause of morbidity and mortality in Nigeria (Alebiosu *et al*., 2006). Acute exposure to toxins has been implicated as one of the underlying causes of renal diseases, (Hakim and Lazarus, 1989, Jacobson, 1991). This study evaluates the possible effect of Sudan IV on the integrity of the kidney.

EXPERIMENTAL

Source of materials/ animals. Thirty-two (32) albino rats (Wistar strain) used for this study were obtained from the animal house of Ambrose Alli university, Ekpoma, Edo State, Nigeria. The feed used were products of Edo Feed and Flour Mills, Limited, Ewu, Edo State. Palm oil was obtained from Palm oil Research Company (PRESCO), Edo State, Nigeria; and Sudan IV, from a standard chemical shop, Silveb Scientifics, in Lagos State, Nigeria.

Treatment of animals. Thirty-two (32) albino rats were divided into four groups of eight rats each. They were acclimatized for 2 weeks on rats' mash and water. The groups were given 90% rats' mash supplemented with 10% palm oil. Sudan IV was administered in the diet to provide levels of 0%, (PO), 0.005%, (PO/0.005), 0.01% (PO/0.01) and 0.015% (PO/0.015). The animals were given these diets for six weeks along with water *ad libitum.* They were weighed weekly; feed intake and faecal output were estimated daily.

Collection of samples. The rats were subjected to an overnight fast, after which they were anaesthetized and blood collected by cardiac puncture into containers with or without anticoagulant. The kidneys were excised, blotted dry and weighed. They were placed in 10% formalin for histopathological analysis.

Methodology. Biochemical analysis was carried out to determine the effect of Sudan IV on total protein, creatinine, urea, sodium and potassium levels in the blood. Total protein, creatinine and urea levels were determined using commercial kits (products of Quimica Clinica Applicada S.A). Creatinine was estimated using the modified Jaffe's method (Spierto *et al*., 1979). At alkaline pH values, creatinine reacts with picric acid to produce a coloured compound, creatinine alkaline picrate, which is photometrically measured. Serum total protein was estimated using the Biuret's method (Peters, 1968); blood urea nitrogen (BUN) by the modified method of Berthelot-Searcy (Fawcett and Scott, 1960). Urea is hydrolysed by urease to produce ammonium ion which on addition of salicylate and hypochlorite, produces a coloured indophenols derivative. Sodium and potassium levels were determined using the flame photometric method as described by Tietz, (1995). Alanine aminotransferase and Aspartate aminotransferase activities were determined based on the colorimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (Reitman and Frankel, 1957). Histopathological analyses

were done using the method of Humason, (1962). The liver tissues were fixed in 10% neutral formalin, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Statistical analysis. All data were expressed as mean ± SEM. One-way analysis of variance was used to test for difference among all the groups. Duncan's multiple range test was used to test for significant differences among the means. P value of < 0.05 was considered statistically significant.

RESULTS

There was no significant (P<0.05) gain in weight in rats administered different levels of the dye compared with control (palm oil diet only), although feed intake were significantly (P<0.05) reduced in the test rats compared with control. There were significant increases in serum creatinine, urea, potassium and ALT levels compared with the control, increases in sodium and AST levels occurred only at 0.01% and 0.015% levels of the dye compared with control. Histopathological examination of the kidney of control and test rats showed increased blood congestion in the veins and thickening of the walls (hypertrophy) of the blood vessels of rats.

Mild accumulation of chronic inflammatory cells in the outer region (cortex) of the kidneys of the control rats was observed (Plate 1). Plate 2, shows the microscopic representation of the kidney of rats administered 0.005% level of Sudan IV dye. There were deposition of fibrous tissue in the inner region of the kidney (fibrosis); these changes were followed with bleeding (haemorrhage) in the outer region of the kidney. Plates 3 and 4 show the microscopic representations of the kidneys of rats administered 0.01% and 0.015% dye respectively. The fibrosis and haemorrhage observed at the 0.005% level were more pronounced at these levels.

DISCUSSION

Investigations conducted to determine the effect of Sudan IV-adulterated palm oil diet on renal function revealed a significant reduction in feed intake of rats administered different levels of the dye compared with the control, although gain in weight (Table 1) observed in these groups did not significantly differ from the control, within the period of administration. This implies a negative impact on appetite. Table 2 shows the effect of the dye on some biochemical indices of renal function.

Table 1: The effects of Sudan IV adulterated palm oil diet on body weight, feed intake and faecal output (g)

Group	Weight gain	Feed intake	Faecal output
PO	30.17±2.1	140.64±4.4	58.04±5.7
PO/0.005	22.33±5.5	121.66±1.1	44.98±3.3
PO/0.01	29.17±7.7	125.42±3.5	50.34±4.4
PO/0.015	30.00±2.3	130.06±4.0	55.32±3.6

Results are expressed as mean ± SEM (n=8) *Significant at P<0.05 Compared with control (PO)

Table 2: The effect of Sudan IV-adulterated palm oil diet on some biochemical indices.

Parameters	PO	PO/0.005	PO/0.01	PO/0.015
Creatinine (μmol/L)	111.07±5.1	148.57±6.2*	157.37±5.8*	183.87±4.1*
Urea (mmol/L)	5.73±0.8	10.0±1.6*	13.7±1.4*	18.5±2.5*
Sodium (mmol/L)	160.40±2.8	158.00±0.7	168.00±3.3*	165.8±1.3*
Potassium (mmol/L)	6.26±0.26	10.45±0.4*	10.15±0.72*	9.88±0.4*
AST(u/L)	27.00±0.49	31.33±1.0	34.00±0.49*	40.33±0.93*
ALT(u/L)	29.17±3.7	43.33±4.7*	40.33±2.3*	45.33±3.7*

Results are expressed as mean ± SEM (n=8) *Significant at P<0.05 Compared with control (PO)

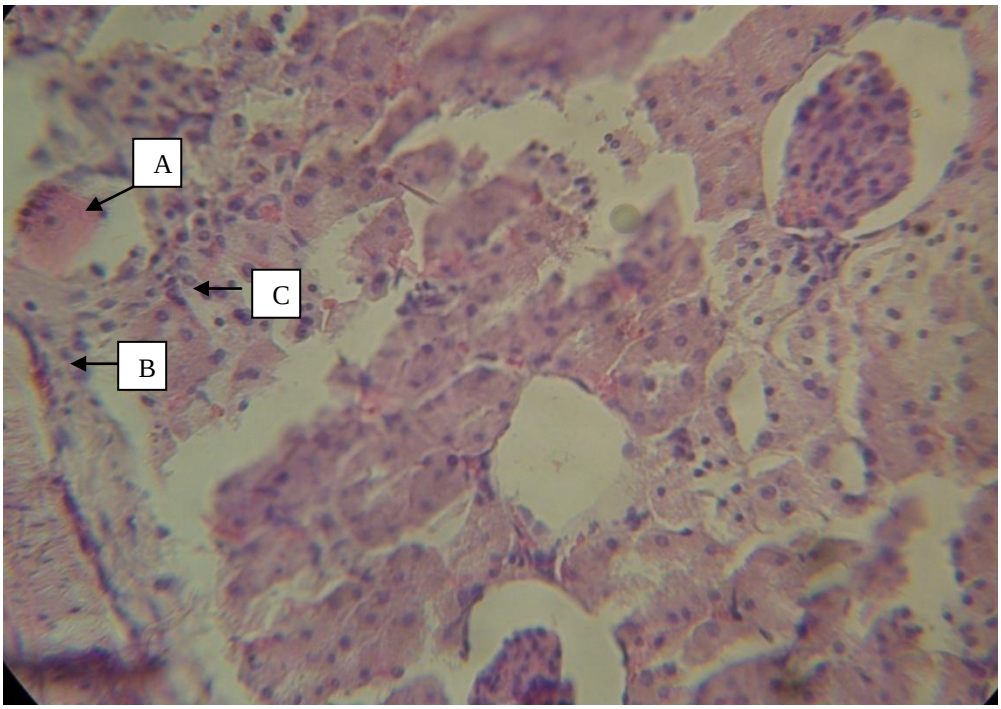

Plate 1: Microscopic representation of the kidney of rats administered palm oil- based diet (control) showing mild congestion (A), vascular hypertrophy (B) and mild infiltrates of chronic inflammatory cells (C) (x10 H&E)

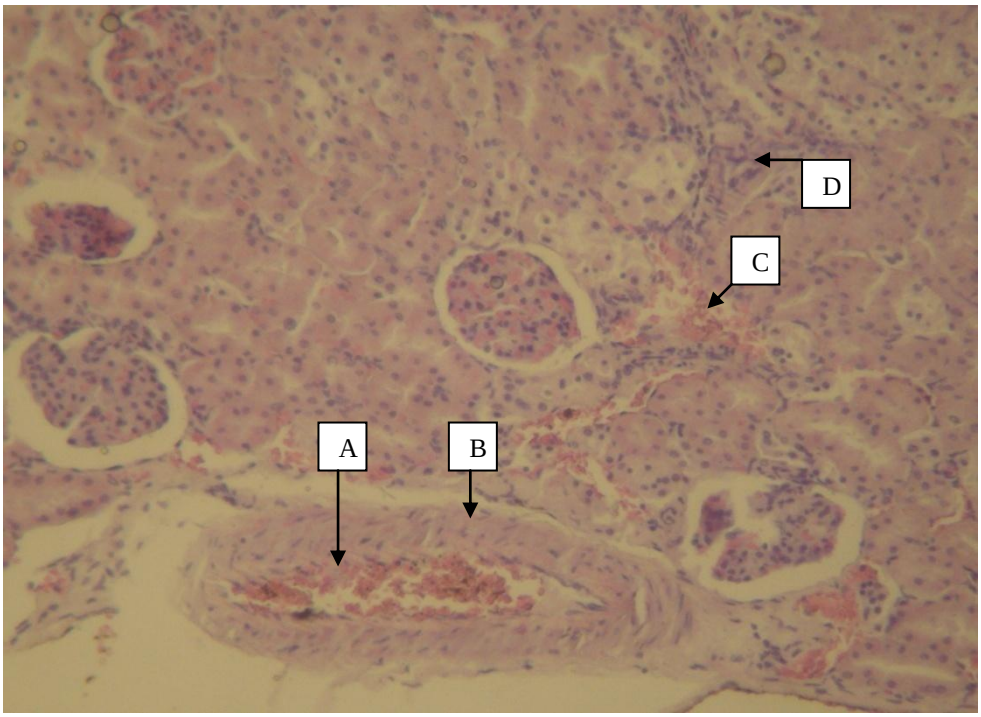

Plate 2: Microscopic representation of the kidney of rats administered 0.005% level of Sudan IV (PO/0.005) showing moderate vascular congestion (A) and hypertrophy (B), mild interstitial hemorrhage (C) and moderate infiltrates of chronic inflammatory cells (D) [x10 H & E)

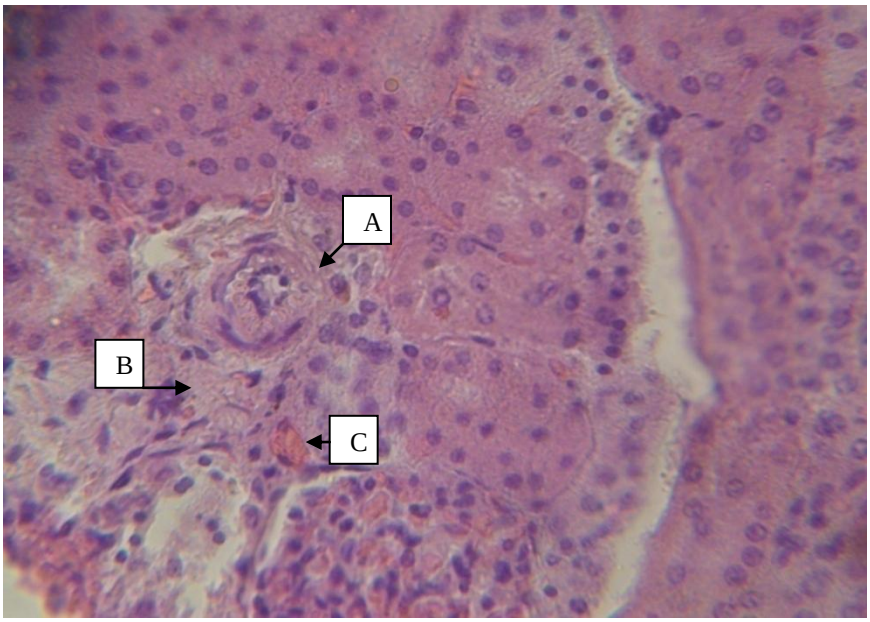

Plate 3: Microscopic representation of the Kidney of rats administered 0.01% level of Sudan IV dye showing focal vascular hypertrophy (A) interstitial hemorrhage (B) and moderate infiltrates of chronic inflammatory cells (C). [X40 H &E).

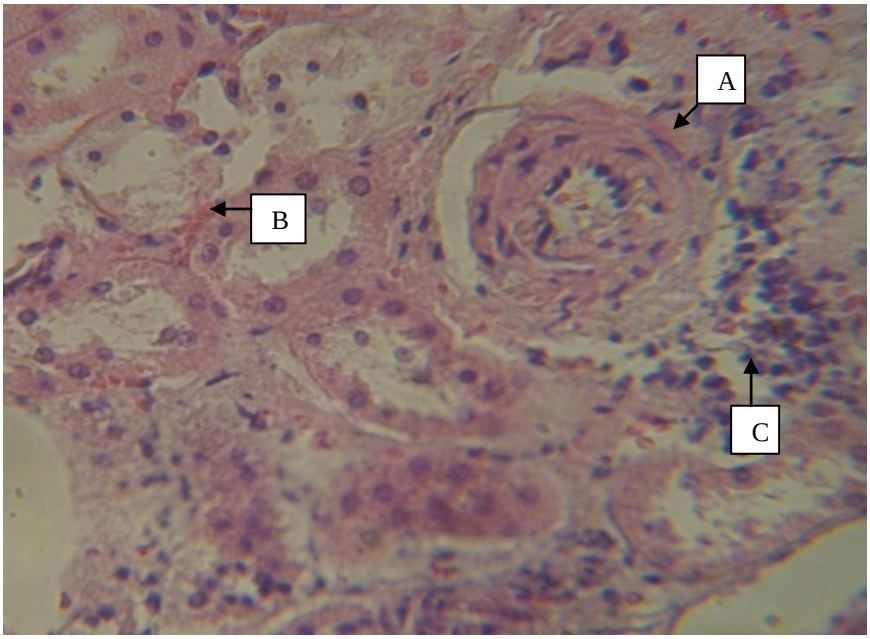

Plate 4: Microscopic representation of the Kidney of rats administered 0.015% level of Sudan IV dye showing focal vascular hypertrophy (A), interstitial hemorrhage (B) and moderate perivascular infiltrates of chronic inflammatory cells (C) [x40 H & E]

Increase in creatinine and urea levels observed signify an alteration in kidney function. Creatinine is produced in muscles when a compound called creatine spontaneously breaks down. Almost all creatinine produced is excreted by the kidneys; so blood levels are a good measure of how well the kidneys are working.

Increased creatinine levels in the blood suggest diseases that affect kidney function; these may include glomerulonephritis (swelling of the kidney blood vessels), pyelonephritis (pus forming infection of the kidneys), and acute tubular necrosis. Destruction of renal mass with irreversible sclerosis and loss of nephrons leads to a progressive decline in glomerular filtrate rate (Arora *et al*., 2006). Similarly, healthy kidneys eliminate more than 90% of the urea the body produces; high urea levels suggest impaired kidney function.

Significant ($P<0.05$) increases in blood potassium and sodium levels were also observed. Hyperkalemia usually develops when the glomerular filtrate rate falls to less than 20 – 25ml/min. because of the decreased ability of the kidneys to excrete potassium (Mailloux, 2001, Lazarus *et al*., 1997). Salt and water excretion is altered in patients with kidney malfunctions; increased sodium levels observed at 0.01% and 0.015% levels may be as a result of kidney malfunction. Decline in kidney function may produce sodium retention and extracellular volume expansion, which may lead to edema and hypertension (Lazarus *et al*., 1997). Alanine aminotransferase (ALT) is found mainly in the liver, but also in smaller amounts in the kidney, heart, muscles and pancreas, aspartate aminotransferase (AST) is normally found in red blood cells, liver, muscles and kidney. Increase in serum levels of ALT and AST observed could also be indicative of kidney malfunction (Paul and Giboney, 2007).

The results of the histopathological examination of the kidney of both control and test rats are presented in Plates 1-4. The results showed that the palm oil based diet (control) provoked vascular activity (inflammation) in and around the blood vessels of the outer region of the kidney, however, with the addition of 0.005% dye (Sudan IV) to palm oil, injury was observed particularly around the blood vessels, with deposition of fibrous tissue. With addition of increasing concentration of red dye, remarkable injury occurred with rupture of blood vessels.

The results of this study reveal a possible deleterious effect of Sudan IV dyes on the kidney at levels often found in adulterated palm oils. Although there is legislation banning the use of this dye in many countries of the world, some developing countries like Nigeria, Ghana, Cameroun and most African countries have problems enforcing these laws. Apart from the well documented mutagenic or carcinogenic effect of this dye, a compromise in the integrity of the kidney has been observed. This may be one of the causes of the reduction in life expectancy of people in some developing countries of the world today.

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