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PHYSIOLOGICAL AND HISTOPATHOLOGICAL STUDY OF SULFANILAMIDE DERIVATIVE ON MALE RABBITS

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ABSTRACT : The experiment was done to examine the effect of sulfanilamide and HSV on hematological and some biochemical parameters in addition histopathological changes in male rabbits, this experiment was carried out on 18 male rabbits, these animal acclimatized for one week before then divided into three groups (6 rabbits in each). First group as control group were given of dimethyl sulphoxide(0.5 ml) I.P daily, second group were treated withsulfanilamide drug (195 mg/kg) I.P daily, while, third group weregivenof HSV(83.86 mg/kg) I.P daily. The treatment continues for three weeks, the results showed significantly reduction in RBC$_s$, Hb, PCV, this reduction was companied by a significant decrease MCH, MCHC, as well as a significant decrease in Platelet count, while observed significantly increase in AST and ALT activities also a significant increase in total cholesterol, the study produced a significant increase in urea and creatininewhereas significantly decrease in total protein and albumin associateda significant increase in globulin, histopathological study showed congested in portal vein and periportal fibrosis, while in kidney dilated cortical tubules and atrophy glomeruli were noted in the male rabbits treated with sulfanilamide, from our results the group treated with HSV showed improvement in hematological and biochemical parameters, while ameliorated the histopathological changes in liver and kidney were showed. Concluded theadministrationofsulfanilamide cause to several adverse effects while the administration of HSV attenuation of this impact.

Key words : HSV, sulfanilamide, hematological, biochemical, histopathological, male rabbits.

INTRODUCTION

Sulfonamides are the first successfully synthesized antimicrobial drugs. The mechanism of sulfonamides antimicrobial action involves competitive inhibition of folic acid synthesis which prevents the growth and reproduction of microorganisms. Due to this mechanism of action, sulfonamides belong to the group of bacteriostatic agents. Sulfonamides are still the drugs of choice for the treatment of several conditions and diseases (Tacic *et al*, 2017).

The sulfonamide has great importance in medicinal chemistry, with various biological activities such as anti-bacterial, hypoglycemic, diuretic, anti-carbonic anhydrase (CA), anti-thyroid *in vitro* and *in vivo*, anti-inflammatory, anti-cancer activities, anti-hypertensive, anti-convulsant (Shoaib *et al*, 2013). Sulfonamides possessing a free amino group are easily derivatizable, leading to a wide range of biomedical applications (Menabuoni *et al*, 1999). The condensation products of sulfa drugs with aldehydes and ketones are biologically active. Schiff bases are used as pigments and dyes, catalysts, intermediates inorganic synthesis and as polymer stabilizers. A number of Schiff's base molecules show biological activities including antibacterial, antifungal, antidiabetic, antitumor, anti-proliferative, anticancer, anti-corrosion and anti-inflammatory activities (Badgujar *et al*, 2015). The current study was aimed to assess the effects of HSV compound and sulfanilamide on hematological, some biochemical parameters and histopathological profile in liver and kidney in male rabbits.

MATERIALS AND METHODS

Experimental animal

Eighteen healthy male rabbits aged 6 months and weights 1000-1600 grams were used and were brought from the local market Basrah city. The animals acclimatized for seven days at the experiment site, animals housed in standard cages maintained under laboratory controlled at temperature 25 ± 2^0C and 12 hours light /dark cycle, food and water *ad libitum* provided daily.

Experimental design

Animals were distributed into 3 groups consisted 6 rabbits in each group as follows:

Control group : six male rabbits were administered 0.5 ml dimethylsulphoxide (DMSO) intra-peritoneal daily for three weeks.

Group 2 : six male rabbits were given 1/20 of LD_{50} (195 mg/kg) of sulfanilamide drug dissolved by 0.5ml (DMSO) intra-peritoneal daily for three weeks.

Group 3 : six male rabbits were received 1/20 0f LD_{50} (83.86 mg/kg) of (HSV) dissolved by 0.5 ml (DMSO) intraperitoneal daily for three weeks.

After treatment for 3 weeks, the samples of blood were collected from the heart, 2 ml of blood was put into a tube containing the EDTA as an anticoagulant for hematological examinations, 3-5 ml of blood was poured into test tube free from an anticoagulant to isolate blood serum for biochemical analysis

Biochemical analysis

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were enzymatically measured by method (Reitman and Frankel, 1957), serum total cholesterol was determined by method (Siedel *et al*, 1983). The serum urea was obtained by method (Tabacco *et al*, 1979), while theserum creatinine was assayed to according method (Wahlefeld, 1974). Total protein and albumin levels were measured by a colorimetric method of Gornall *et al* (1949), the serum globulin was calculated by the formula: Serum globulin = Serum total protein – Serum albumin.

Hematological parameters

Blood parameters were measured by the using Auto Hematology Analyzer BC-5300, which included RBCs count, Hb, PCV, MCH, MCHC and platelets count.

Histological study

After three weeks; animals were sacrificed and specimens from the organ (liver and kidney) were excised immediately and fixed in 10% buffered formalin, after being fixed the tissues were dehydrated in a series of alcohol concentration (ethanol), then treated with xylene and embedded in paraffin. These tissues were mounted on glass slides, de-waxed and stained with hematoxylin and Eosin (H & E). The section tissues were examined using X10 and X40 objected for histological changes (Luna, 1993).

Statistical analysis

In this study, ANOVA analysis one way (IBM SPSS, version 20) program was used for analysis of results of the present study. The data were expressed as mean ± standard deviation (mean ± SD). Least significant difference test (LSD) was used to test the difference between means (groups); $P \leq 0.05$ was considered significant (SPSS, 2001).

RESULTS

As shown in Table 1, there was a significant decrease ($p \leq 0.05$) in the number of RBC, Hb concentration, PCV and platelet count in male rabbits treated group with sulfanilamide when compared with control, while in group treated with HSV showed significantly ($p \leq 0.05$) increase in the RBC, Hb, concentration, PCV and platelet count compared with treated group with sulfanilamide.

According to the Table 2 the results showed in treated group with sulfanilamide, there was a significant decrease ($p \leq 0.05$) in MCH and MCHC concentrations, at same group there was a significant ($p \leq 0.05$) increase in AST, ALT and total cholesterol levels in treated group with sulfanilamide in compared with control group. While, treated group with HSV produce a significant ($p \leq 0.05$) increase in MCH and MCHC concentration, in addition the administrated of HVS produce significantly ($p \leq 0.05$) decrease in AST, ALT and total cholesterol levels the effect compared with treated group with sulfanilamide.

Depending on the results clarified in Table 3, there was a significant ($p \leq 0.05$) decrease, total protein and albumin concentrations and a significant ($p \leq 0.05$) increase in urea, creatinine and globulin concentration in treated group with sulfanilamide in compared with control. The findings in same table showed that there was a significant ($p \leq 0.05$) increase in total protein and albumin concentrations whereas a significant ($p \leq 0.05$) decrease in urea, creatinine and globulin concentration in treated group with HSV when compared with treated group with sulfanilamide.

The histopathological findings showed congested portal vein, moderate periportal fibrosis of the liver in the treated group with sulfanilamide in Fig. 2 compared with

Table 1 : RBCs count, hemoglobin concentration (Hb), packed cell volume (PCV) and platelets count in control and treated groups.

Parameters / Groups	RBC count × 10^6 cell/mm^3	Hb g/100ml	PCV%	Platelet count × 10^6 cell/mm^3
Control group 0.5 ml DMSO	5.84 ±0.47a	12.41±0.88a	41.13±1.10a	638.27±8.64 a
Sulfanilamide drug (195 mg/kg)	2.89±0.11 c	7.16 ±0.47c	23.79±1.39 c	372.90±13.83 c
HSV (83.86mg/kg)	4.30 ±0.40b	10.50 ±0.55b	33.98±1.41 b	496.71±34.26 b

Mean ± SD (n = 6). The different letters refer to significant difference compared with control at level of ($p \leq 0.05$).

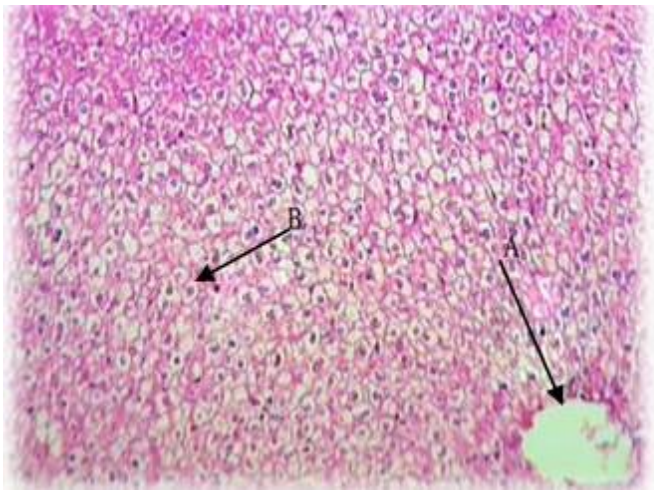

Fig. 1 : Liver from the control group showed normal central vein (A) and hepatocyte (B) H &E X 100.

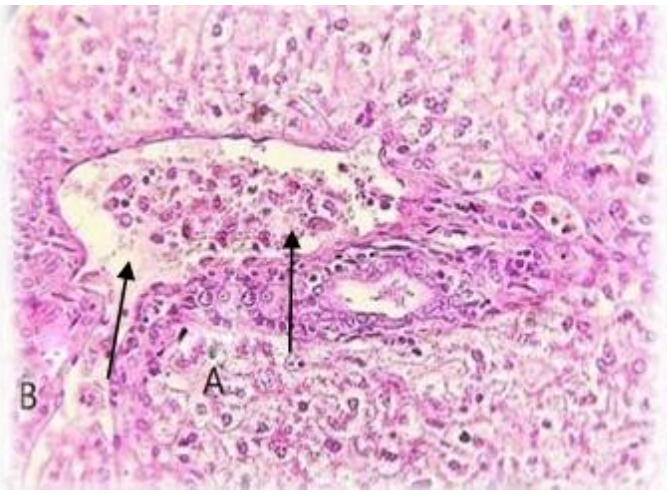

Fig. 2 : Liver from the group treated with sulfanilamide showed congested portal vein (A) moderate periportal fibrosis (B) H and E X 400.

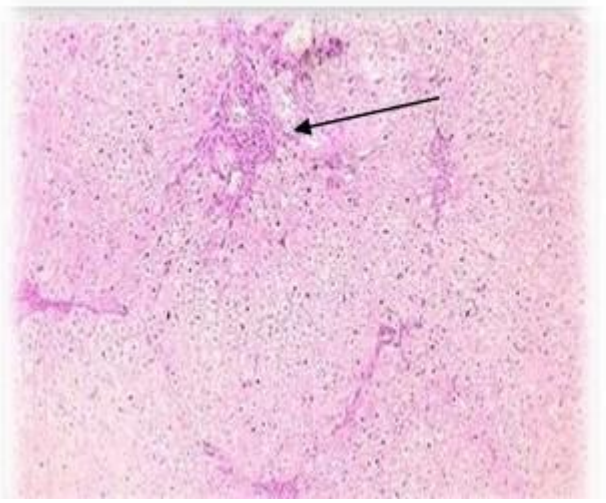

Fig. 3 : Liver section from the group treated with HSV showed minimal periportal fibrosis (black arrow) H and EX100.

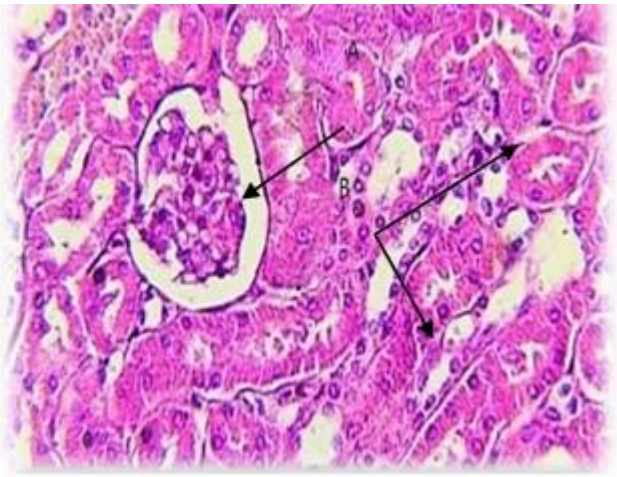

Fig. 4 : Kidney section of the control group showed normal glomerulus (A) and renal tubules (B) H and EX 400.

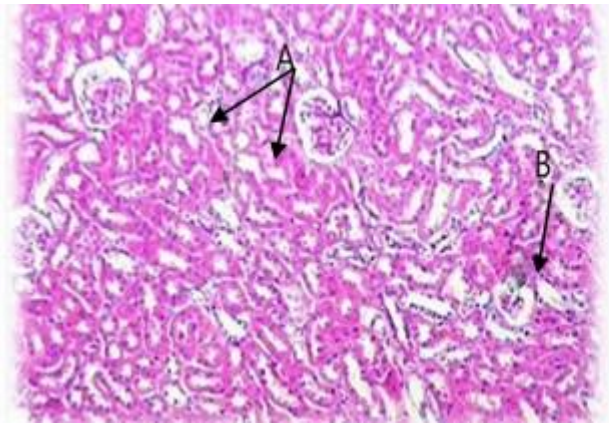

Fig. 5 : Kidney section from the treated group with sulfanilamide showed dilated cortical tubules and atrophy of the glomerulus (B) H and E X100.

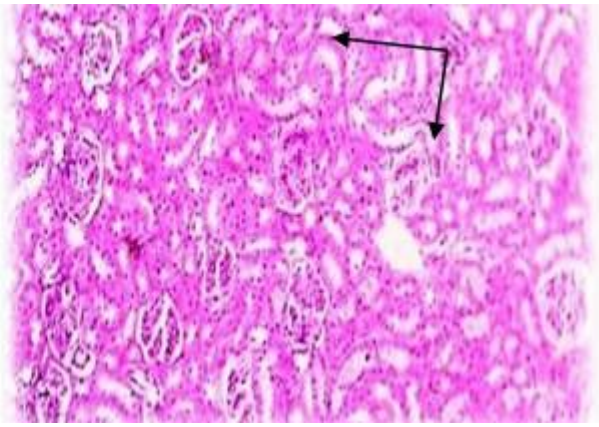

Fig. 6 : Kidney section from the treated group with HSV Showed a number of glomeruli appear nearly normal (black arrow) H and E X100.

Table 2 : Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration(MCHC), alanine transaminase (ALT), aspartate transaminase (AST) and total cholesterol (TC) in control and treated groups.

Parameters / Groups	MCH(pg)	MCHC%	AST unit/L	ALT unit /L	TCmg/dl
Control group 0.5 ml DMSO	41.49 ±4.90 a	38.28 ±1.40a	32.34±2.24 c	60.50±3.49 c	88.99±1.69c
Sulfanilamide drug (195 mg/kg)	26.30±1.83 c	20.99 ±1.82 c	48.73 ±2.02 a	87.78±1.20a	137.89 ±1.68a
HSV (83.86mg/kg)	34.95±2.15 b	31.09 ±1.45 b	39.37 ±1.43b	69.08 ±1.65b	98.01±1.08 b

Mean ± SD (n = 6). The different letters refer to significant difference compared with control at level of ($p \leq 0.05$)

Table 3 : Serum urea, creatinine, total protein, albumin and globulin in control and treated groups.

Parameters / Groups	Urea mg/dl	Creatinine mg/dl	Total protein mg/dl	Albumin mg/dl	Globulin mg/dl
Control group 0.5 ml DMSO	26.22±1.63 c	0.79 ±0 .11c	5.93±0.86 a	4.36±0.24a	2.21 ±0.08 c
Sulfanilamide drug (195 mg/kg)	38.37±1.16a	1.38 ±0.02a	4.17 ±0.12c	3.03±0.07 c	3.83±0.30a
HSV (83.86mg/kg)	29.12±1.38b	1.04 ±0.04b	5.28 ±0.06b	3.60±0.10 b	2.93±0.11 b

Mean ± SD (n = 6). The different letters refer to significant difference compared with control at level of ($p \leq 0.05$).

the control group in Fig. 1. While, the liver of rabbits treated with (HSV) showed minimal periportal fibrosis in Fig. 3. Whereas, the alteration in the kidney with sulfanilamide treatment showed dilated cortical tubules and atrophy of the glomeruli in Fig. 5 as compared with the control group in Fig. 4. While, the kidney of the group treated with HSV showed the number of glomeruli appear normal limit in Fig. 6.

DISCUSSION

The results of the current study in Tables 1, 2 showed changes in the blood parameters in rabbits treated with sulfanilamide induced a significant decrease ($p \leq 0.05$) in RBC count, Hb, PCV, MCH, MCHC and platelet count compared with control due to the immune hemolytic anemia caused by sulfanilamide (Gehrs and Friedberg, 2002). These results in agreement with other studies (Mintzer *et al*, 2009; Aster *et al*, 2009 and Tacic *et al*, 2017) showed a significant decrease in complete blood count when treated with drugsdue to these drugs more commonly cause mild marrow suppression and inducing aplastic anemia which characterized by pancytopenia with a hypo-cellular bone marrow like sulfonamide. While, the results presented in the same tables showed a significant increase ($p \leq 0.05$) in blood parameters (RBC, Hb, PCV, MCH, MCHC and platelet count in the treated group with (HSV) due to the protective effect of (HSV) to the blood parameters, which decreases lipid peroxidation on the blood cells membrane as well as membrane fragility (Makni *et al*, 2012). This results in agreement with Tai *et al* (2011), who showed that vanillin has much stronger antioxidant activity than ascorbic acid, which is reacted with radicals *via* a self-dimerization mechanism. The data in Table 2 showed a significant increase ($p \leq 0.05$) in AST, ALT and total cholesterol levels in the treated group with sulfanilamide in compared to control due to the adverse effect of sulfanilamide on the liver, which causes an immunoallergic reaction that causes severe hepatocyte injury and it is an important target of toxicity to oxidative stress and toxic chemicals such as antibiotics, chemotherapeutics and carbon tetrachloride (Lee, 2003 and Singh, 2017). Similar finding with Verma and Kaplowitz (2009), who showedliver is the target organ for sulfanilamide metabolism, liver damage lead to increasing of ALT and AST enzymes into the extracellular fluid and plasma and increased total cholesterol level. The results in same Table 2 showed the effect of HSV on ALT, AST and total cholesterollevels that there was a significant decrease ($p \leq 0.05$) ALT and AST activity and total cholesterol levelin the treated group with HSV due toimproving effect of HSV on the liver the results agreed with other studies (Srinivasan *et al*, 2008; Raja and Mol, 2010; Belagali *et al*, 2013 and Saad *et al*, 2016) found the ability of vanillin to ameliorate the adverse effects on hepatic tissues, thus vanillin inhibited damage via its antioxidant activity and inhibit the oxidation of LD and hypolipidemic influence of vanillin in induced hypercholesterolemic and hypertriglyceridemic rats. From our results in Table 3 pointed out that there was a significant increase in urea, creatinine and globulin levels and a significant decrease in total protein and albumin levels in the treated group with sulfanilamide agreed with several studies (Rossert, 2001; Kodner and Kudrimoti, 2003; Singh *et al*, 2003, Schetz *et al*, 2005 and Perazella and Markowitz, 2010) found that this elevation in these parameters due to the harmful effect of sulfanilamide on the kidney that many drugs cause nephrotoxicity to have toxic effects by one or more common pathogenic

mechanisms. These medications that cause acute interstitial nephritis are thought that bind to antigens in the kidney or act as antigens that are then deposited into the interstitium, inducing an immune reaction. According to the data in Table 3 showed that a significant decrease ($p \leq 0.05$) in urea and creatininelevels and a significant increase ($p0 \leq .05$) in albumin concentration in the treated group with (HSV) due to the charitable effect of HSV compound, which have less harmful effect on renal tissues than sulfanilamide. This current results agreed with Saad *et al* (2016), Elseweidy *et al* (2017), who pointed out in their studies, the protective effect of vanillin on the kidney. Histopathological changes are associated with biochemical alteration, the effects of sulfanilamide are very obvious and deleterious on liver and kidney which confirmed with central vein congested and moderate periportal fibrosis in the liver and dilated cortical tubules and atrophy of glomeruli in the kidney, similar finding agreed with other studies (Naughton, 2008; Odigie, 2013; Singh, 2017) showed the administration of sulfanilamide causes degeneration of hepatic cells and causing renal impairment. These changes confirmed by a significant increase liver enzymes ALT and AST activity which resulted and increase ($p \leq 0.05$) in urea, creatinine and globulin levelswhile, a significant decrease ($p \leq 0.05$) in total protein and albumin levels. Whereas the administration of HSV showed ameliorated these changes, this due to beneficial effect of HSV compound on the liver and kidney functions.

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