Histopathological Study of Sub- Acute Toxiciy Induced by Relife in Mature Male Rats

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Abstract

The present work was conducted to study the sub-acute toxicopathological changes of relief in mature male rats . Thirty male rats aged (10) weeks and weighted (60 -120) gm were divided equally into (2) groups as follows: Group I: Rats served as control group and received distilled water for 30 days . Group II: Rats served as experimental group and received (by gavage 500 mg /kg b.w) one tablets of relief (composed of Diclofence sodium 50mg , paracetamol 500 mg ,chlorpheniramine malete 4 mg and magnesium trisilicate 100 mg) diluted in 10 ml of distilled water . Specimens from, liver, kidneys, testes, epididymis ,brain and spleen, were dissected out for histopathological examination. Results showed severe histopathological changes in all selected tissues as a results of sub acute toxicity of relief tablets.

Keywords: relief, sub- acute toxicity , rats , histpoathological changes.

الخلاصة

هدف العمل الحالي الى دراسة التغيرات الامراضية السمية الحادة للتسمم بامراض الرليف في ذكور الجرذان البالغة . استخدم في الدراسة (30) جرذ وبعمر (10) اسابيع واوزان تراوحت بين (60–120) غم حيث قسمت مجموعتين متساوتين وكما يأتي: المجموعة الاولى جرعت ماء مقطر لمدة 30 يوم وعدت مجموعة سيطرة، المجموعة الثانية جرعت 500ملغم /كغم من وزن الجسم من قرص واحد للرليف (يتكون من ديكلوفينات الصوديوم 50 ملغم ,الباراسيتامول 500 ملغم ,كلورومفينيرامين ماليت 4 ملغم و المغنسيوم ثلاثي السليكات 100ملغم) مذاب في 10مل ماء مقطر.تم اخذ عينات من والكبد والكلية و الخصى و البربخ و الدماغ و الطحال لغرض اجراء الفحوصات المرضية النسجية.اوضحت النتائج حصول تغيرات امراضية نسجية قوية في الاعضاء الماخوذة نتيجة للتسمم تحت الحاد للرليف.

الكلمات المفتاحية :الرليف ,التسمم تحت الحاد ,جرذان ,التغيرات الامراضية النسجية .

Introduction

Relief is a drug widely used in treatment of headache, body ache, fever, toothache and gout pain around the world, and available without prescription .These drug is composed of (Diclofence sodium 50 mg, paracetamol 500 mg, chlorpheniramine malete 4mg and magnesium trisilicate 100 mg). Paracetamole and diclofence sodium were developed specifically as a non-steroidal anti- inflammatory (NSAID) drugs. It competes with arachidonic acid for binding to cyclo-oxygenase (COX), resulting in glucoronidation decreased formation of prostaglandins. (Vane and Botting 1996; El Maddawy and El-Ashmawy, 2013). Both drugs are metabolized in liver and after glucoronidation and sulphation the metabolites are extracted in the urine and bile (Bin, *et al* .,2009).The drugs cause severe damage in most of the vital organs

in the body including kidney ,liver, brain ,heart and testes (Tomic,2008; Majeed *et al*., 2013; Jum *et al* .,2015). The key characteristics of acetaminophen poisoning include the development of necrosis of the liver cells characterized by eosinophilic degenerations, in addition,to that polymorphonuclear cells infiltration has also been reported in these cases. (Kelvin *et al*., 2015).chlorpheniramine malate is described as histamine H_1 receptor or antagonist , is an antihistaminic of the alkylamine group .This drug widely uses in cattle ,goats ,pigs and horses ,also used in humans. Chlorpheniramine malate has a variety of other actions particularly in CNS (transient hypotension or stimulation)(European agency for the evaluation of medical product, 1999). Chlorpheniramine malate rapidly absorbed after oral administration ,and it is the major metabolite in urine of dog and rats . In rats the highest tissue concentration were observed in lungs, kidney and liver and significant level were also detected in brain and muscles . (Tella and Owalnde, 2007). Magnesium tricilicates antiacids are used for the treatment of heartburn, dyspepsia, gastritis, and gastroesophageal reflux,with prolonged administration and/or excessively large doses. Dysrhythmias, hypo- and hypertension, encephalopathy, acute renal failure, gastrointestinal obstruction and/or perforation metabolic alkalosis, fluid, electrolyte, and minera derangements, and myopathies and osteodystrophies have been reported. Distention and/or obstruction with perforation and hypergastrinemia have been observed with prolonged or excessive antiacid administration (Rumack,2016).Nonsteriodal antiinflammatory drugs which are mediated by inhibition synthesis of prostaglandin, It have been involved as a regulation of several physiological processes in human body such as inflammatory processes in immune response, vasodilator, vasoconstriction, pain perception and fever. Prostaglandin is produced in every tissue of the body (brain, lung, kidney, intestinal digestive system, male and female reproductive system) (Fahar *et al* ., 2016).

Material and Methods

Chamicals

Drug

Relief tablets (each tablets composed from Diclofence sodium 50 mg , paracetamol 500 mg ,chlorpheniramine malate 4mg and magnesium trisilicate 100 mg) were obtained from Xianhe Pharma Company ,China .

Animals

Thirty male rats were used for the present study. The animals were housed in metal cages in the animal house of the Veterinary Medicine College, University of Alqasim green and were fed on standard rat pellets, with water provided *ad libitum*, they were allowed to acclimatize for 10-14 days at room temperature.

Experimental design

Thirty male rats aged (10) weeks and weighted (60 -120) gm were divided equally into (2) groups as follows: Group I: Rats served as control and received distilled water

for one month. Group II: Rats served as experimental group and received by gavage 500 mg /kg b.w of the drug for 30 days.

Histopathological Study

At the end of experiment period ,the animals of each group were sacrified by intramuscular injection of high dose of ketamin hydrochloride. Specimens were taken from, liver, kidneys, testes , epididymis , brain and spleen, the tissues were fixed in 10 % formaldehyde solution then processed routinely by using the histokinette. Tissue sections were embedded in paraffin, sectioned by microtome and stained with hematoxylin and eosin (13).

Results

Histopathological study

Control group:showed normal histological structures for all organs involved in experiment .

Treated group

Liver :

The liver showed extensive area of necrosis with marked dilation in sinusoids, dilation and congestion of central vein, also an increased in the number of kupffer cell and focal infiltration of lymphocyte beside blood vessels (fig 1) .

Kidney : the main lesion was diffused tubular degeneration and interstial hemorrage (fig 2) .

Testes

The histopathological changes in the testes included necrosis, (fig 3) apoptosis, (fig 4) and vacoulation in seminefrous tubules. The germinal layer lining the seminefrous tubules showed slouhging in their epithelia and degranulation .Severe congestion of main testicular and interstial blood vessels (fig 3) .Degeneration of leydic cells with tubular atrophy ,the seminefrous tubules appeared shrinked and has irregular membranes (fig 5) .

Epidedymus

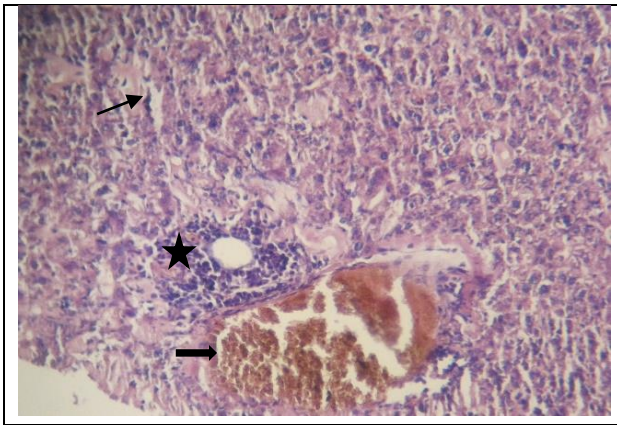
The epidedymus showed som tubules empty from sperm ,and others have small ammount of mature and immature sperm cells. Fibrosis like apperance was also seen in som region with congestion of blood vessles(fig 6).

Brain :

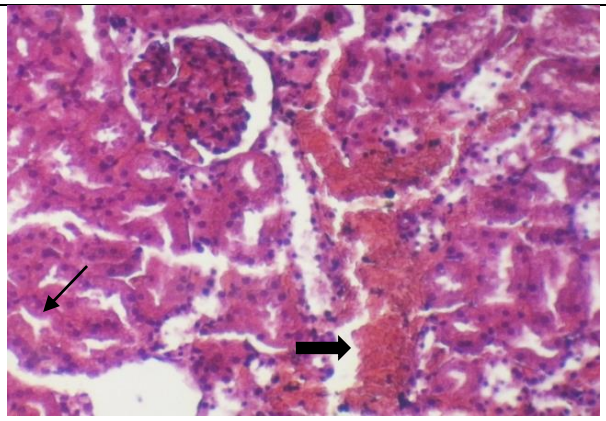
There was vacoulation arround the neurons due to cerebral odema, (fig 7) .increase in the number of astrocytes and focal glaiosis with gaint cells formation(fig 8) , congestion in cerebral blood vessels also was seen (fig 9) .

Spleen :

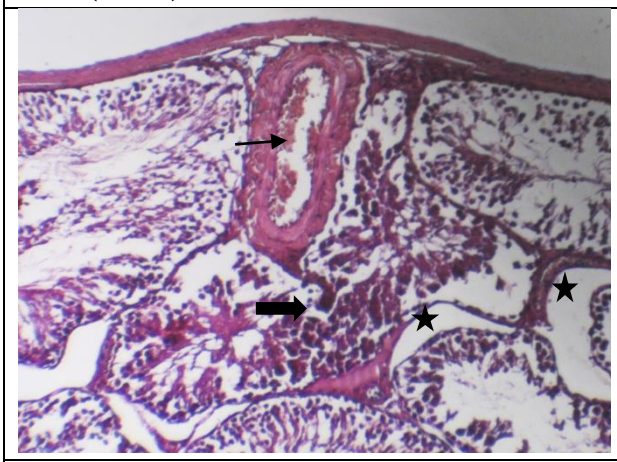
There were severe depletion in lymphoid follicles (white bulb), with marked hemmorage with hemosidrin laden macrophage also seen (fig10).



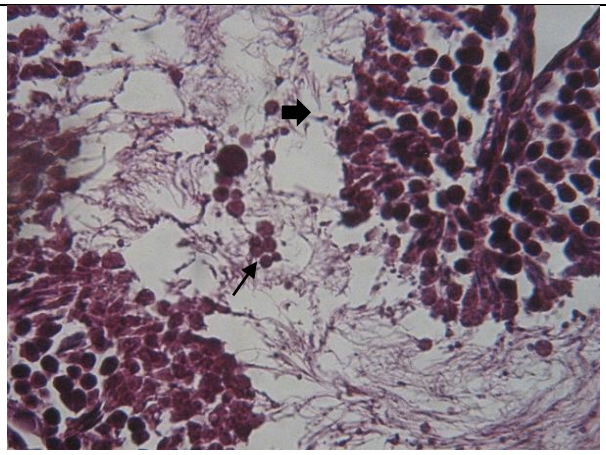
2:Histopathological section of rat liver showing centrilobular necrosis (➙) ,with dilation and congested central vein (➡),and focal infiltration of inflammatory cell beside the central vein (★). H&E (400x).



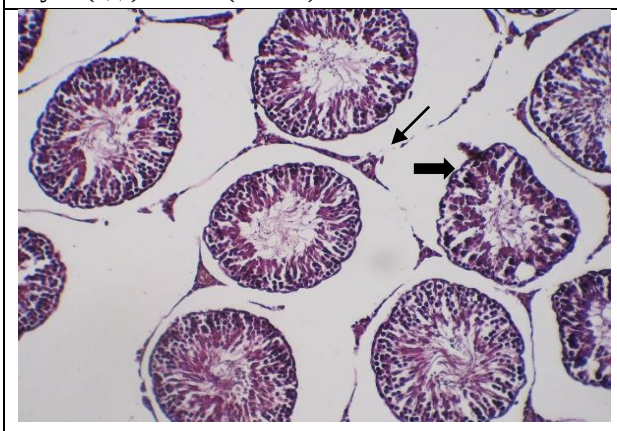
2:Histopathological section of rat kidneys showing degeneration of renal tubules(cloudy swelling (➙)with interstital hemorrhage (➡) .H&E (100x).



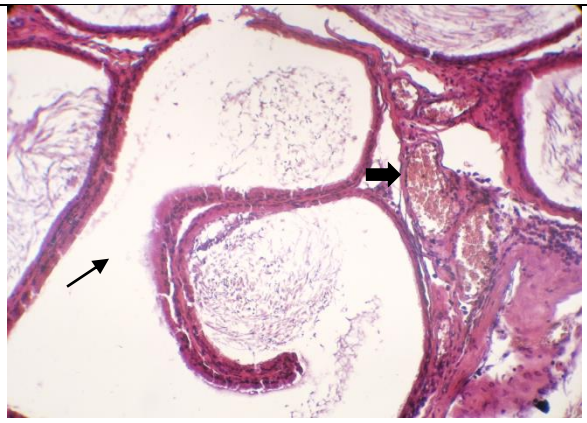
3:Histopathological section of rat testes showing congestion of testicular blood vessels (➙). With tubular necrosis (➡) .degranulation of germinal layer (★) H&E (400x).



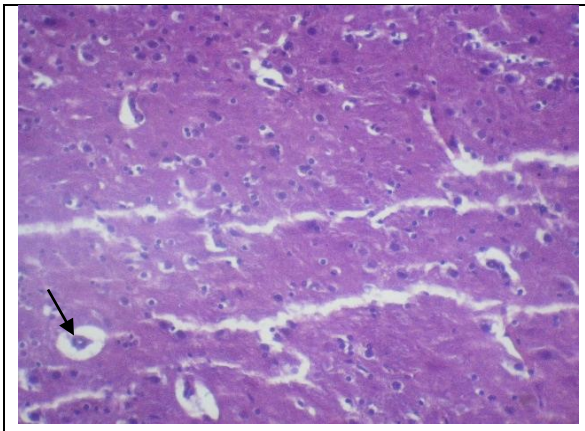
4:Histopathological section of rat testes showing apoptosis (➙) and immature spermatozoa (➡) . H&E (400x).



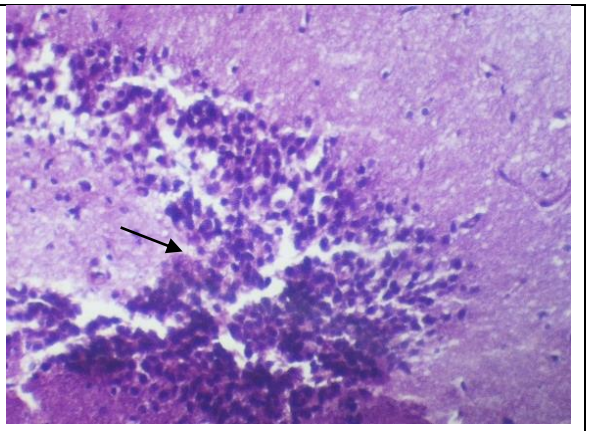
5:Histopathological section of rat testes showing atrophy of seminiferous tubuleswith irregular membranes (➡) and degeneration of leydic cells ( ) . H&E(400x)



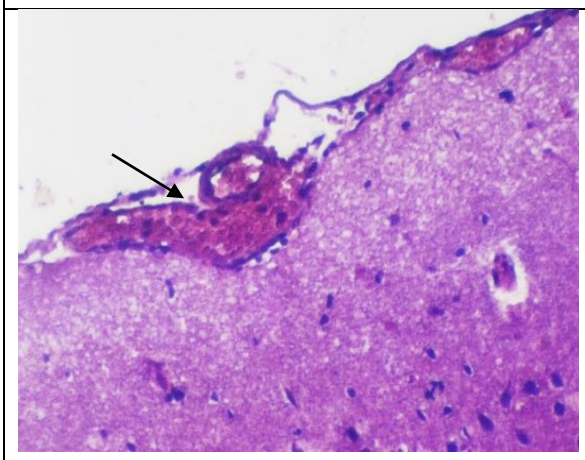
6:Histopathological section of rat epididymis showing severe dilation of epididymis tubules (➡), with congestion blood vessels (➙) . H&E (400x).



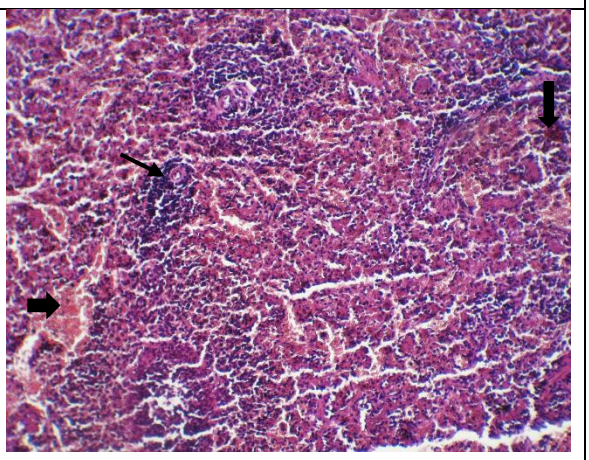
7:Histopathological section of rat brain showing cerebral edema (➔. H&E(100x).



8:Histopathological section of rat brain showing focal gliosis (➔. H&E(100x).



9:Histopathological section of rat brain showing congestion of cerebral blood vessels (➔) . H&E(100x).



10:Histopathological section of rat spleen showing depletion of lymphoid follicles (⬇) with sever hemorrhage (➡ and hemosidrosis (➔) . H&E (100x).

Discussion

Non-Steroidal anti-inflammatory drugs are the most frequently prescribed therapeutic agents, used for treatment of rheumatic diseases, , because they have rapid analgesic, antipyretic and anti-inflammatory actions.(Zeynab *et al*.,2013) .The results of the present study showed extensive area of necrosis and degenerative kidney and liver with inflammatory cells infiltration in liver .This changes occur due to the active compound of this drugs which may be cause distraction of ATPase activity in mitochondrial plasma membrane leading to cellular damage (Ruepp *et al* .,2002).This results agree with (Nelson ,1999;Kelvin *et al* .,2015) whom postulated that "mitochondrial proteins could be primary cellular targets to acetominophens leading to the loss of activity of energy production in the cells ". Or it may be due to oxidative stress which promote cells death (James *et al* .,2003). Wongnawa *et al*., 2002 and Majeed *et al* .,2013 described the primary mechanism of paracetamole toxicity by saying that biotransformation of paracetamole via cytochrome P-450 that lead to produce ROS (superoxide anion radials , H_2O_2,OH^-) leading to oxidative stress .ROS

enhance lipid peroxidation and lead to membrane damage and necrosis (Dezwart *et al*., 2002; Suchittra and Sorrayut, 2014). Gujral *et al* .,2002, established that" paracetamole overdose can cause liver injury and even failure in both humans and animals .Cell death occur as a result of apoptosis and necrosis . In a study using 300mg/kg of paracetamole for induction of hepatotoxicity ,it was shown that apoptosis cells death was present at two hours after paracetamole administration ,also the number of necrotic cells correlated with the increase in the activity of ALT in the liver "(Kalvein *et al* .,2015).

Diclofence sodium also cause liver ,kidney ,testes, lung and gastrointestinal toxicity (Brater,2002; Tomic *et al*.,2008) ,This toxicity was related to the drug metabolism and was reduced by the addition of cytochrome P-450 inhibitors to the culture media (Boelsterli ,2003).Mitochondrial damage and decrease it NADpH are also thought to be responsible for diclofence hepatotoxicity (Masubuchi *et al*.,2002).In a study conducted by Zeynab *et al* .,2013 showed that "administration of rats of diclofence sodium in rats at dose 13.5 mg /kgb.wt for 2 weeks induce marked increas in lipid peroxidation ,malondialdehyde (MDA) content in liver and kidney tissues and decreased GSH in both tissues ". the metabolism of diclofence sodium increased the generation of ROS these products induce prooxidative damage in renal tissue (Gokcimen *et al.,* 2000,2001) .

The brain tissue is more susceptible to damage that caused by ischemia and oxidative stress and ,the histopathological results of the present study showed cerebral edema ,congestion of cerebral blood vessels and focal microgiliosis . These changes may be attributed to the ability to relief to cross blood brain barriers (BBB) efficiently, and the disruption of the blood brain barrier (BBB) function leading to disturbance in blood dynamics and the escape of the fluid to the nervous tissue or it might be primarily related to damaged permeability of BBB. (Bernareggi,1993; Parepally *et al* .,2006;Marial *et al* .,2010) . This result agree with previous studies that reported that NSAIDS cause oxidative stress in brain tissue and a decrease in glutathione level (Nencini *et al* .,2007 :Scorticai *et al* .,2004 ;Ilic *et al* .,2010). NSAIDS also cause reduce in PGE_2 synthesis by inhibiting the activity of COX with in the CNS (Greco ,2003;Anderson ,2008) .

The results showed that treatment with relief drug for one month cause depletion in lymphoid follicles and intestinal hemorrhage in spleen which may occur due to the composition of this drug which gives antiinflammatory properties that cause a decrease in leukocyte recruitment. This result agree with (Kouya *et al* . ,2003). Who reported that " nonsteroidal anti-inflammatory drugs may down regulate Th1 and, to a lesser extent, Th2 immune responses and proliferation of spleen cells to the antigen." Hemorrhage occur due to vascular changes in the splenic arterioles and damage of splenic tissue followed by lysis of RBCES to form hemosiderin that engulfed by microphages .(Lukey and Petersen (2001). The testis is the most important organ in male reproductive system. It is responsible for two main functions, synthesis of steroid hormones and production of spermatozoa (Vyas *et al* .,2016). Relief cause various effects in testicles and epididymis including necrosis and apoptosis and slouphing in

seminefrous tubules with vacoulation in some region which may be attributed to the ability of relief to produce the reproductive toxicity through disruption of epithelial cell function or might be by acting directly on spermatozoa by affecting their enzymes (Al-Inany *et al* .,2016). Apoptosis occur as a result of decreased in testosterone level as a result of degeneration in leydic and sertoli cells that lead to increase in germ cell apoptosis which lead to decreased reproductive ability(El-Sharaky *et al* .,2014), or it may occur due to disturbance of microenvironment of the Sertoli cells, that affect the protein synthesis essential for differentiation of germ cells, (Nasiraei *et al* .,2014).Also the drug cause degeneration in leydic and sertoli cells with tubular atrophy and shrinking in seminiferous tubules with irregular basement membranes .This changes might be due to the effect of drug to on spermatogenesis, among these effects are the effects of the chemical component of drugs and toxic elements of environmental pollution (Walker,2009). There are many pathological lesion detected in testicular and epididymis tissues including the germinal layer lining the seminefrous tubules which showed slouhging in their epithelia and degranulation .severe congestion of main testicular and interstial blood vessels and degeneration of lydic cells with tubular attrophy ,the seminefrous tubules appeared shrinked and has irregular membrane. The epididymis tubule was empty of the spermatozoa and contain small numbers of mature and immature sperm cells. Fibrosis like apperance also seen in som region with congestion of blood vessels.Thes Nonsteriodal antianiflamatory drugs produce physiological, cytotoxic and genetic abnormalities which might be lead to alterations in testicular DNA that disrupts the process of differentiation of spermatozoa (Movahhedin and Vaez Mahdavi, 2014). Exposure to chemicals could produce pituitary-hypothalamic effects which could affect spermatogenesis, and resulting in functional or structural impairment of sperm cells (Ekaluo *et al* .,2008), decreased Sertoli and leydic cells number and distubtion in collagen of basment membranes resulted reduce testicular size (Marettova *et al* ., 2015 ; Li *et al* ., 2016).

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