ABSTRACT

The present study was designed to evaluate the feasibility and safety of Phenobarbital on partial hepatectomy operation in local goats. Sixteen adult local breed goats, both sexes, aged between nine month to one year and weight ranged between 25-35 kg, were studied, which divided randomly into four equal groups: A- Partial hepatectomy with Phenobarbital injection of 80 mg/kg daily for 45 days. B- Partial hepatectomy without Phenobarbital injection for 45 days. C- Partial hepatectomy with Phenobarbital injection of 80 mg/kg daily for 90 days. D- Partial hepatectomy without Phenobarbital injection for 90 days. The results showed that partial hepatectomy could be performed successfully in goats. The complications that occurred in the present study included the following: adhesion with omentum site of hepatectomy on the 45 and 90 days of Phenobarbital groups but it was adhesive of liver with diaphragm in groups that were not treated on 45 and 90 days. Liver regeneration appeared early and was extensive on 45 and 90 days of Phenobarbital groups treatment, through the congestion, central vein, present portal trail, compact hepatocytes, compared to other groups without treatment. Testes showed suppression of spermatogenesis in all groups treated with Phenobarbital compared to other groups. Finally, we can conclude that Phenobarbital had a positive effect on the partial hepatectomy. It is the best for the regeneration of hepatocytes, the proliferation of bile duct, but it had suppressive effects on spermatogenesis. The aims of this study, Insure that Phenobarbital has a regenerative effect on liver after hepatectomy.

KEYWORDS: Toxicology, Phenobarbital, Hepatectomy & Goats
INTRODUCTION

The Phenobarbital is a barbiturate and it is the most widely used anticonvulsant worldwide [1]. It is also used to control epilepsy (seizures) and as a sedative to relieve anxiety. It is used for short-term treatment of insomnia to help to sleep, [2]. It was used to treat neonatal jaundice by increasing liver metabolism and thus lowering bilirubin levels.[3]. The liver is a vital organ presents in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis and production of biochemicals necessary for digestion. This organ plays a major role in metabolism and has a number of functions in the body.[4]. Partial hepactectomy is the surgical removal of part of the liver. The purpose of hepatectomies performed is to surgically remove tumors from the liver. Most liver cancers start in liver cells called "hepatocytes", the resulting cancer is called hepatocellular carcinoma or malignant hepatoma. [5]. There are always risks with any surgery, but a hepatectomy that removes 25-60% of the liver carries more than the average risk, pain, bleeding, infection and or injury to other areas in the abdomen, death is a potential risk, as well. Other risks include postoperative fevers, pneumonia and urinary tract infections. Patients who undergo any type of abdominal surgery are also at risk of forming blood clots in their legs. These blood clots can break free and move through the heart to the lungs.[6]. Barbiturates are extensively metabolized by the liver. The plasma clearance of barbiturates can be decreased and the half – lives prolonged in patients with impaired hepatic function. Therapy with barbiturates should be administrated cautiously and initiated at reduced dosage in patients with liver disease [7].

MATERIALS AND METHODS

Animals

Sixteen healthy adult local breed goats were selected from a herd animals of both sexes, aged between nine months to one year and weight ranged between 25-35 Kg, resource of the veterinary medicine college –Basrah University. All goats were treated against internal and external parasites by using Ivermectin (Kela-Belgium)at dose of 200 µg/Kg B.W. subcutaneously with Rafoxind 3% at a dose 1.25 mg /kg B.W. The animals were fasted for 36 hours of food and 12 hours water with held preoperatively.

Experimental Animals

The total numbers of 16 goats were divided randomly into 4 main groups, each group contain (three male and one female) as follows:
1- **First group: group (A)**, Partial hepatectomy with Phenobarbital injection intramuscular of 80 mg/kg daily for 45 days.

2- **Second group: group (B)**, partial hepatectomy without Phenobarbital injection for 45 days.

3- **Third group: group (C)**, partial hepatectomy with Phenobarbital injection intramuscular of 80 mg/kg daily for 90 days.

4- **Fourth group: group (D)**, partial hepatectomy without Phenobarbital injection for 90 days.

**Histopathological Technique**

The specimens were taken from animals after necropsy on the 45 and 90 days, these include (thyroid, Salivary glands, duodenum, nerve, pancreas, kidney, testes, epidymus and liver). Then fixing in natural buffered formalin (10%), dehydration was done by passing the specimens in increasing concentration of ethanol, infiltrated 3 times with xylene or chloroform and embedded in baraffin. 5-6 micrones and stained with Hematoxyline – Eosin. and finally examined under light microscope. [8].

**RESULTS**

**Histopathological Findings**

The histopathological findings of the liver after partial hepatectomy were as following:

**Fourty five days postoperation**

Specimens goat from group (A) after 45 of treatment showed congestion of central vein and presence of portal traid including portal vein, portal arterial and bile duct. The portal vein was dilated and congestend while sinusoid were dilated (figures-1,2,3). Minimal periportal fibrosis with hypertrophy was seen in addition to the proliferation of bile duct epithelium. Mark regenertionin the area of the operation associated with marked fibrosis in the area Solid masses of regenerated liver cells also appeared. Sinusoid can be hardly seen, but no evidence of neoplasia, just normal physiological regeneration of hepatectomy foci of mix inflammation cells (fig:4). Compact, regenerated hepatectomy hepatocytes. With (minimal vaculation (fig:5). The liver seen compact the hepatocytes with minimal cytoplasm and non hypertrophic (fig: 6) The epithilium of the bile duct was proliferation and
hypertrophied (fig:7). Specimens taken from the testes showed suppression of spermatogenesis, vacuolation of spermatogenic and sertoli cell (figures - 8 and 9). Seminiferous tubule, showed vacuolation of spermatogoni and sertoli cells indicating suppression of spermatogenesis(fig-10). The pancreas showed vacuolation and degeneration with prediabetic changes in the islets of langerhans (fig-11). Specimens goat from group B 45 days post operation showed moderate fibrosis, regeneration, hypertrophy of hepatocytes, moderate bile duct proliferation and hypertrophy moderate periportal fibrosis (fig-12). Also the liver showed the moderate to marked periportal fibrosis, vacuolation area and congestion per portal vein (fig-13). The testes containing primary and secondary spermatogenesis with spermatozoa in lumen (fig - 14).

**Ninety Days Postoperation**

Specimens obtained from group C 90 days after treatment compact hypertrophic hepatocytes sinusoids were not prominent (Fig-15). Proliferation of bile duct, hypertrophic of hepatocytes (Fig-16). Also circumscribed proliferation and regeneration of hepatocytes was also detected. The partial hepaectomy, and subcapsular fibrosis can be noted (fig-17). In compact, a periportal area of fibrosis, bile duct proliferation and congestion can be seen. There was clear evidence of proliferation and regeneration of hepatocytes with minimal centrilobular vacuolation of hepatocytes, also because of liver cell proliferation sinusoid could be hardly noticed. There was moderate fibrosis at the site of hepaectomy (fig–18). The fibrosis and large bile duct with hypertrophic proliferation epithelium can be seen (fig-19). The subcapsular fibrosis in addition to septal fibrous in (figure – 20). The testes shows suppression of spermatogenesis, vacuolation of spermatogonia and primary spermatocytes, spermatogenesis to only primary spermatocytes (fig-21,22). The pancreas showed vacuolation of Islet of langerhans, due to toxic effect of Phenobarbital (fig-23,24). Specimens goat from group D 90 days post the operation without treatment of Phenobarbital. shows moderate periportal fibrosis, moderate periportal mixed inflammatory cell, congested portal vein, and proliferation of bile ducts (fig:25,26,27). Testes seminiferous tubules show evidence of spermatogenesis (Fig- 28).
Figure (1): The histological section of liver of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing the congested central vein (CV), portal traid (PT) dilated congestion portal vein (Co). H&E stain 20 x

Figure: (2): The histological section of liver of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing dilated the portal veins (PV) and bile ducts (BD). H & E stains 20 X
Figure: (3) : The histological section of liver of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/dairy) showing congestion (con) with dilated sinusoids (Si) with focus of mononuclear cell. H & E stains 20x

Figure: (4) : The histological section of liver of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing peri portal fibrosis (pf) peri portal (per) and parenchymal foci mixed inflammatory cells (pa.fo) congestion portal veins (con). H & E stains 20x
Figure: (5) : The histological section of liver of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing compact regeneration hepatocytes (He.co) hardly sinusoids(si) and minimal vacuolations (vac) H & E stains 20x

Figure: (6) : The histological section of liver of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing compact Hyper-trophic hepatocytes (hy) and minimal cytoplasmic vacuolation (cy).H & E stains
Figure: (7) : The histological section of liver of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing hypertrophic (bil) proliferated epithelium (epith). H & E stains 40x.

Figure: (8) : The histological section of testis of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing interstitial oedema (ed) and suppression of spermatogenesis (sper). H & E stains 4x.
Figure: (9) : The histological section of testis of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing sertoll cells (ser) and suppression of spermatogenesis (sp) H & E stains 10x

Figure: (10) : The histological section of testis of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing seminiferous tubules (sem) with vacuolation of spermatogonia (vac) and sertoli cells indicating suppression of spermatogenesis (ser) H & E stains 40x
Figure (11): The histological section of pancreas of group (A) partially hepatectomized treated with phenobarbital 45 days (80mg/daily) showing the islets of Langerhans (lan) and vacuolation indicating degeneration with prediabetic changes (ser) H & E stains 40x

Figure (12): The histological section of liver of group (B) partially hepatectomized without treated with phenobarbital 45 days (80mg/daily) showing the hypertrophy of bile duct with inflammatory cells (per) moderate peri-portal fibrosis (fib) and dilatation with congested bile duct (con) H&E stain 20x
Figure: (13) : The histological section of liver of group (B) partially hepatectomized without treated with phenobarbital 45 days (80mg/daily) showing the moderate to mark peri-portal fibrosis (per) vacuolation area (voc) and congestion perportal vein (con) H&E stain 20x

Figure: (14) : The histological section of testis of group (B) partially hepatectomized without treated with phenobarbital 45 days (80mg/daily) showing primary and secondary spermatogenesis (sper) and spermatozoa in lumen (sp) H &E stains 40x.
Figure: (15): The histological section of liver of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing the liver is compact of hepatocytes (he). H&E stain 20 x

Figure: (16): The histological section of liver of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing the liver is proliferation bile ducts (bi), circumscribed area of hypertrophy and proliferated hepatocyte (HE) and fibrosis (fi). H&E stain 40 x
Figure: (17): The histological section of liver of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing the liver is area of capsular fibrosis (fi). H&E stain 20 x

Figure: (18): The histological section of liver of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing the liver is compacted hepatocytes (co) and congestion of the bile duct (con) also proliferated the bile ducts (prol). H&E stain 20 x
Figure: (19): The histological section of liver of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing the liver is proliferation of hepatocytes (prol) and large bile duct (bil) with proliferated epithelium preportal and fibrosis (fi). H&E stain 40 x

Figure: (20): The histological section of liver of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing the liver is septal fibrosis (sep) and sub capsular fibrosis (fi). H&E stain 40 x
Figure: (21): The histological section of testis of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing primary spermatogenesis (pri) indicating suppression of spermatogenesis (sp) and vacuolation (vac) of spermatogonia and serotil cells H & E stains 40x

Figure: (22): The histological section of testis of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing primary spermatogenesis (pri) suppression of spermatogenesis (sp) and oedema (oed) H & E stains 40x
Figure: (23) : The histological section of pancreas of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing the islets of langerhans (lan) and vacuolation (vac) H & E stains 40x

Figure: (24) : The histological section of pancreas of group (C) partially hepatectomized treated with phenobarbital 90 days (80mg/daily) showing the islets of langerhans (lan) and vacuolation (vac) H & E stains 40x
Figure: (25): The histological section of liver of group (D) partially hepatectomized without treated with phenobarbital 90 days (80mg/daily) showing the moderate peri-portal mixed inflammatory cells (inf) moderate peri-portal fibrosis (fib) and proliferation of bile ducts (bil) H&E stain 20x

Figure: (26): The histological section of liver of group (D) partially hepatectomized without treated with phenobarbital 90 days (80mg/daily) showing the moderate peri-portal (fi) fibrosis (inf) H&E stain 20x
Figure: (27): The histological section of liver of group (D) partially hepatectomized without treated with phenobarbital 90 days (80mg/daily) showing the moderate peri-portal (fi) proliferation of bile ducts (pro) H&E stain 40x

Figure: (28): The histological section of testis of group (D) partially hepatectomized without treated with phenobarbital 90 days (80mg/daily) showing seminiferous tubules with normal H & E stains 40x
DISCUSSION

We have previously shown that various Phenobarbital, could include a state of hyperplasia, although the literature about this subject may be considered controversial. Data of histopathological of changes this study are extended and compared to previous work. Histopathological sections in group A showed the evidence in the inflammatory cells and fibrosis around the central vein, this idea was agrees with [9]. But the changes in group B is normal in anything about inflammatory cells and congestion as well as fibrosis and proliferation bile ducts. These observations were described by other researches [10&11]. Microscopic finding observed at each group in all animals that they were similar to treated groups but differ from untreated ones due to the effect of the Phenobarbital specially in liver, testes and pancreas. But the compact of hepatocytes is very clear in the 90 days treatment with Phenobarbital due to the Phenobarbital stimulation of SER and effect the CY 450 of biotransformation. This agree with [12] who explain that the hepatocyte, regeneration occur when injury affecting hepatocytes with stromal tissue and also agrees with [13]. The hyperplasia in bile duct response seems to be linked with other part of the liver, that agrees with [14]. This study tested several hypotheses about the effects of Phenobarbital on liver regeneration, testes and pancreas due to the androgen sensitive. Reproductive organs of treated animals suffering from suppression of spermatogenesis through the effect of Phenobarbital such as necrosis of the testicular cells that agree with [15]. Treatment of goat with Phenobarbital induce the level of glutathione S-transferase D in testes with no increase in the activities of glutathione S-transferase A and C. This result indicate a specific induction of Yb2 subunit in testes as well as degenerative changes in sertoli cells which are well supported with biochemical studies that provide the effect of Phenobarbital in transference with the maturation process of testes. That also agrees with [16]. The Phenobarbital effect on the testes through decreasing the action of endogenously producing androgens by stimulating their metabolism agrees with [17]. There is over production of one type of cells; these crowds out and decrease the production of other cells types, Phenobarbital treatment increases the concentration of cytchrme P-450 in treated groups to the same extent. However greater CYP2B/2B2 activity was found in control goats following Phenobarbital administration. Little is known about the biochemical alterations that underlie cellular autolysis, it has been assumed by many that pronounced tissue acidosis, such as by causing release of activation of lysosomal enzymes, can cause irreversible cell damage.[18] Repeated Phenobarbital doses causes a large increase in NADPH –cytochrom C reductase amount nonconcomitantly with a
moderate rise in cytochrome b5 content, both effects appear to result from a drastic reduction in rates of enzymes degeneration. Phenobarbital affects regulatory mechanism by which the cell controls its content of certain membranes and membrane-bound enzymes. The net effect is a proliferation of smooth surface endoplasmic reticulum membranes, and concomitantly a large increase of some, but not all, of the enzymes associated with the type of membrane in the normal cells. Phenobarbital is metabolized by the enzymes system whose increase in amount it induces, and repeated injections of the drugs are needed to obtain maximal effects which have been extensively studied. Our findings also show that an even earlier event than the increase in rate of reductase synthesis is an increase in the rate of incorporation of amino acid into total microsomal at 3-4 hours after a Phenobarbital injection, that agrees with [19].

Hepatocytes, which are normally quiescent in the adult animal, are readily induced to proliferate by partial heptectomy. All of extracellular signals that medicate regenerative process act initially at the plasma membrane, where binding of receptors to endocrine and paracrine agent elements of extracellular matrix, transport of nutrients, and intracellular with neighboring cells occur. That agrees with [20].

REFERENCES