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POTENTIAL IMPACT OF TIME ON DNA DAMAGE, MICRONUCLEUS WITH LIVER CONTENTS AND FUNCTION AFTER PARTIAL HEPATECTOMY IN RATS

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AUTHORS' CONRTIBUTIONS

This work was carried out in collaboration between both authors. Authors AAE and SMG designed the study, managed the literature searches and analyses of the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Liver regeneration after partial hepatectomy (PH) is one of the most captivating phenomena in medicine. The aim of this work was to study the effect of time (days) on DNA damage, micronucleus, liver contents (weight, water and lipid %) and function after PH in male rats was evaluated. Liver weight, water and lipid contents as well transaminases enzymes, bilirubin (BR) and protein were determined. In addition, DNA fragmentation, micronucleus (Mn) induction and hemoglobin were also assessed. Liver weight was decreased significantly and restored to normal by day 3. However, lipid content was increased significantly but restored to normal by day 7. Water content was similarly increased in regenerating liver and restored the normal by day 28. Transaminases, Brill and protein levels were decreased and restored to normal by day 14. In addition, hemoglobin was decreased and restored to normal by day 28. DNA fragmentation and micronucleus frequency were increased significantly compared to control (day 0) and restored to normal on day 7 and 28 for DNA and Mn, respectively. In conclusion, liver regeneration in male rats after 70% partial hepatectomy (PH), response at 3rd, 7th, and 14th days. Our results might be of clinical relevance for human medicine.

Keywords: Liver regeneration; Partial Hepatectomy (PH); DNA fragmentation; function; liver contents; rats.

ABBREVIATIONS

PH : Partial hepatectomy
MN : Micronucleus
AST : Aspartate aminotransferase
ALT : Alanine aminotransferase
ALP : Alkaline phosphatase
BR : Bilirubin
Hb : Hemoglobin
PODs : Postoperative days
RR : Regeneration rate
Cdk : Cyclin-dependent kinase
SDS : Sodium Dodecyl Sulphate
SD : Standard deviation

1. INTRODUCTION

Liver cancer is one of the leading causes of cancer-related death worldwide [1-3]. According to the data from International Agency for Research on Cancer, the global incidence rate of liver cancer continues to rise, for example, more than 222,000 cases were reported in the United States in 2016 [4]. Despite recent advances in diagnosis and treatment, liver cancer remains difficult to treat and has a high mortality rate. Patients diagnosed with early-stage liver cancer can be treated by local surgical resection or liver transplantation [5].

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Liver regeneration is one of the most captivating phenomena in medicine that has fascinated clinicians, surgeons, and scientists who have observed this apparently supernatural process and studied its mechanisms for many years. The liver is the largest internal organ and possesses multiple substantial functions in the human body. It plays an important role in the homeostasis of carbohydrate, protein, and lipid metabolism. It is responsible for synthesis and storage of glycogen from glucose through glycogenesis [6].

The liver is characterized by complex physiology and the ability to undergo rapid regeneration [7-9]. Liver regeneration starts as a response to different types of liver tissue damage, including partial hepatectomy (PH) with removal of 70% of the liver. Liver regeneration occurs by a compensatory hyperplasia and hypertrophy of the residual liver lobes, not involving stem cells [10]. This means that liver regeneration following acute injury like PH arises within hours from the remaining hepatocytes and not from stem cells [6,11].

Loss of liver tissue in vertebrates is followed by the re-entry of the remaining hepatocytes into the cell cycle, DNA-replication, and cell division, a process termed compensatory hyperplasia [11], which eventually results in restoration of the original liver mass. This process has been extensively studied in rodents which underwent surgical removal of approximately two thirds of the original liver mass (partial hepatectomy, PH) [12].

The restoration of resected hepatic tissue is a complex process involving cellular hypertrophy and hyperplasia, during which profound alterations of synthetic and differentiated functions of the liver occur. The morphologic and biochemical events occurring from initiation to completion of this process have been studied. The phenomenon of hepatic regeneration is directly involved in human pathology (e.g., cirrhosis [13] and the recovery phase of hepatitis [14]). By assessing liver weight and regeneration rate (RR) dynamics, high hepatocyte proliferation on postoperative days (PODs) 1-3, after which levels fell to nearly zero on day 5 was found by [15], who concluded that, a 70% PH in healthy rats induces a rapid regenerative response and PODs 2, 4 and 8 seems optimal for assessing hepatic growth in future studies. In addition, water and lipid contents of regenerating rat liver after 70% hepatectomy was determined by Sostman et al. [16], who found that lipid content of tissue samples, was increased in regenerating tissue but not in controls. In addition, water content was similarly increased in regenerating liver tissue until the day 13 after PH. They attributed this alterations in portal blood flow following resection of 70% of the liver account for the changes in water content.

Following PH in the rat, hepatocyte DNA synthesis peaks abruptly at 24 h after PH, and essentially terminates DNA synthesis by 72 h following resection [7].

Unchanged hepatic DNA content was found in all hepatectomized and control groups and liver RNA content was higher in hepatectomized rats (21%, $P < 0.05$) than other group. Also, hepatic protein synthesis was significantly increased in all hepatectomized groups compared with their respective control [17]. Regeneration of liver protein mass can be achieved by 2 mechanisms, either by increased protein synthesis or by decreased protein breakdown [18].

There are more than 20 types of transaminases that are the important indicators for liver function. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (BR) are the most commonly used clinical indicators for the evaluation of liver function [19]. The alteration of ALT, AST, ALP and BR following liver injury may be associated with trauma and changes in intraoperative blood flow and have an important role in the evaluation of liver function following surgery [20]. Therefore, the present work was designed to study the time onset of liver regeneration response after 70% partial hepatectomy (PH) in male rats, on DNA damage, micronucleus induction, liver contents (weight, water and lipid %) and function.

2. MATERIALS AND METHODS

2.1 Animals and Partial Hepatectomy

Adult male Westar rats weighing 230 to 250 gm were obtained from the animal house of the National Research Center, Dokki, Giza, Egypt. The animals were housed in an environment illuminated 12 hr/day, maintained at approximately 25°C. Laboratory food and water were supplied *ad libitum*. Animals were bred, maintained and treated according to the Guide for the Care and Use of Laboratory Animals (NIH, 1996) [21]. The approval number is: 20 130. Seven groups (10 animals/each) were sacrificed by cervical dislocation at 0, 1, 2, 3, 7, 14 and 28 days following 70% hepatectomy during ether anesthesia, using the method of Mitchell and Willenbring [22], with minor modifications. Briefly, general gas anesthesia was induced and maintained by a mixture of O2 and N2O (0.5 l/min and 1.5 l/min) and isoflurane (Is of loH, 4% for induction and 2% for maintenance). All

interventions were performed under sterile conditions, while body temperature was kept on 37 uC using a heating pad with feedback control by an intrarectally placed sensor. For PH, the abdomen was opened via a midline skin and lina alba incision. The median and left lateral lobe were ligated and resected. The resected liver specimen was immediately weighed in order to estimate liver mass. After PH, 1 ml 0,9% NaCl was given intraperitoneally, the lina alba closed by simple continuous suture pattern using 3/0 absorbable sutures materials, while the skin closed by simple interrupted sutures pattern using 3/0 nonabsorbable sutures materials. Next, rats were allowed to recover from anesthesia under heat-producing lamps. Samples of regenerated liver tissue were immediately removed, briefly stored on ice, weighed and subjected for further analysis.

2.2 Determination of Weight, Water and Lipid Content in Liver

The preoperative total liver weight was calculated from the resected liver weight [12 cited in 15]. Postoperative total liver weight was measured at sacrifice. The change in liver weight was evaluated as the hepatic regeneration rate (RR). RR is defined as (liver weight per 100 g of the body weight at sacrifice/preoperative projected liver weight per 100 g of the body weight) × 100:

$$\mathrm{RR} = \frac{(\mathrm{LWm}/100\mathrm{gBW})\ \mathrm{sac}}{(\ \mathrm{LWp}/100\mathrm{gBW})\ \mathrm{pre}} \mathrm{X}\ 100$$

LWm is the measured liver weight at sacrifice; LWp is the preoperative projected liver weight.

Water content was determined by calculating the difference in weight before and after drying the tissue at 110°C for 2 hrs.

2.3 Determination of Lipid Content

The dry tissue resulting from water content determination was soaked in petroleum ether (40-60) (El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt) for 1 day then in diethyl ether for another day. The tissue was removed and dried at 110°C for 2 hrs. The lipid content was calculated as the difference in weight of tissue before and after exposure to solvents. In addition, change in weight of the remains liver was determined as percentage of total body weight.

2.4 Micronucleus Assay

Liver MN assay was performed according the method of [23]. Briefly, Pellets of freshly washed hepatocytes were resuspended in about 0.5 ml standard medium per tube. With a regular Pasteur pipette two drops of cell suspension were then transferred to a regular microscope slide supplied with filter paper having a hole and a sedimentation chamber on top of it. Just before the cells became dry the slide was transferred to a staining jar and was further treated as follows: Stand slide for 1 h at room temperature in a fixation mixture of methanol, glacial acetic acid and 35% formaldehyde (85 : 5 : 10 vol. parts), then stained and subjected to MNs counting.

2.5 DNA Fragmentation

Liver was homogenized in lysis buffer containing 50 mM Tris–HCl (pH 7.5), 10 mM EDTA and 0.5% sodium dodecyl sulphate (SDS), and incubated overnight with proteinase K (200 µg/ml) at 50°C. After RNase digestion, DNA was extracted and electrophoresed on 2% agarose gel as previously described [24,25].

2.6 Biochemical Analysis

Blood was sampled from the heart at sacrifice. Serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (BR) and total protein were measured using the Modular P (Roche Diagnostics, Mannheim, Germany).

2.7 Statistical Analysis

Values were expressed as mean ± SD (standard deviation). Statistical differences of means were performed by independent samples, one-tailed "t" test. Comparisons were performed by Spearman's rank correlation. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Liver Weight

Mean weight of the resected 70% liver was 2.8658 g, giving an estimated mean total liver weight (100%) of 4.094 (Table 1 & Fig. 1). Gain in liver weight during the recovery/regenerative period for each group was observed on day 3 (1.271±0.182*** vs. $3.94\pm0.2398^{N.S}$ gm, for day 0 and day 3, respectively). Major loss was noted during days 0-2 and surpassed normal preoperative weight on day 3 before returning to baseline on day 7.

3.2 Water Determination

Compared to controls, the water content of the regenerating liver samples increased at all times

studied following surgery (Table 1 & Fig. 1) until the 14-day group. Although slight, the differences were statistically significant. For liver, the peak of increase (75.655±1.660*** compared to 70.224±0.258 and70.738±0.483$^{N.S}$ for day 2 compared to day 0 and 28, respectively) in water content occurred at 48 hr after surgery.

3.3 Lipid Determination

Table 1 & Fig. 1, marked alterations occurred in extractable lipid in regenerating liver tissue compared to control livers, there was about 2 fold increase in lipid at 1 day, and at 2 days, and about 126% increase at 3 days, all differ significantly from control. By 7 days, extractable lipid had returned to normal controls.

3.4 Mn, DNA Fragmentation and Hemoglobin

Results of Mn frequency are presented in Table 2 and Fig. 2, a significant increase in the frequency of MNs from day 1 up to day 14 postoperative was found compared to day 0 and restored to normal control on day 28. As well, DNA fragmentation increased significantly from days 1-3 and decline from days 7-14 and reached to normal control level on day 28. Hemoglobin level, decreased significantly from days 1 -14 before returning to normal control on day 28.

3.5 Transaminases and Protein Levels

Table 3 and Fig. 3, showed the Transaminases (AST, ALT, ALP, BR) and protein levels from day 0 to day 28 groups. The AST concentration was significantly decreased during the early part of the postoperative period with a level on day 1 of 21.48±1.163*** U/L compared to50.646±1.050 on day 0, and increase by the following days (2 -14 days) before reaching a steady state of approximately 51 U/L on day 28. The ALT concentration was significantly decreased during the early part of the postoperative period with a level on day 1 of 21.951±1.529*** U/L compared to 52.158±1.325 on day 0, and increase by the following days (2 -14 days) before reaching a steady state of approximately 52 U/L on day 28.

Alkaline Phosphatase (ALP) showed a significant decrease during the first day (149.948±14.396*** compared to 288.947±18.978 U/L) on day 0. Afterwards, a rising slope was observed, approaching a steady state of approximately 288 U/L on day 14 and 28. Bilirubin (BR) demonstrated a pattern similar to alkaline phosphatase with a significant decrease on day 1 upto day 7 compared to day 0 (0.398±0.20, 0.127±0.022***, 0.213±0.018***, 0.234±0.021***, 0.289±0.019**, for days 0, 1, 2, 3 and 7, respectively). The level on day 14 was 0.369±0.030 $^{N.S}$ umol/L and the steady state level was approximately 0.390 umol/L on day 28.

For protein level (Table 3) there was a statistically significant decline in protein to 50% of the initial level at day 1 after PH (13.181±0.554 and 7.4250±1.183*** g/dL, for day 0 and day 1, respectively). This decline was maintained until day 7 after PH. Afterwards, a rising slope was observed on day 14, approaching a steady state of approximately 13 g/dL on day 28.

4. DISCUSSION

The present study conducted with [16], have demonstrated marked alterations in water, lipid, weight and protein content of regenerating rat liver. The liver water and lipid content were significantly increased in hepatectomized male rats. The same findings were reported by [16], who attributed that alterations in portal blood flow [26], following resection of 70% of the liver account for the changes in water and lipid contents. Moreover, liver weight decreased significantly by days 0-2 and increased significantly and restored its normal weight by day 3 and more after PH. That coincide with [15] who concluded that, a 70% PH in healthy rats induces a rapid regenerative response and PODs 2, 4 and 8 seems optimal for assessing hepatic growth in future studies.

The loss of liver tissue is followed by the re-entry of the remaining hepatocytes into the cell cycle, DNA-replication, and cell division, a process termed compensatory hyperplasia [7,11], which eventually results in restoration of the original liver mass and this support our findings. Following PH in the rat, hepatocyte DNA synthesis peaks abruptly at 24 h after PH, and essentially terminates DNA synthesis by 72 h (day 3) following resection is in consistent with [7]. However, unchanged hepatic DNA content in all hepatectomized and control groups in rats was reported by [17].

Cyclins and cyclin-dependent kinase (Cdk) complexes have important regulatory roles during cell cycle progression [27,28]. PH was found to accelerated degradation of existing protein and this down-regulation occurs precisely within the interval between surgery and onset of DNA synthesis which supports the hypothesis that it mediates activation of G0/S-phase Cdk/cyclin-complexes and re-entry of hepatocytes into the cell cycle [29].

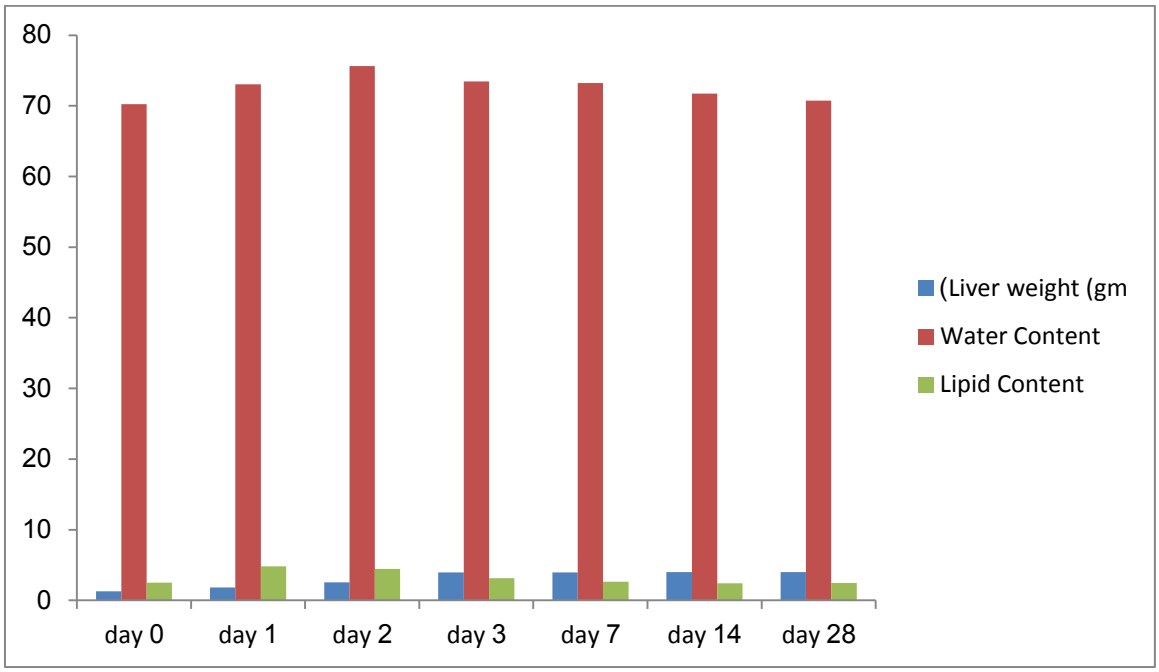

Fig. 1. Effect of time (days) on weight, water and lipid % in rat liver after partial hepatectomy (mean ± SD)

Table 1. Effect of time (days) on weight, water and lipid % in rat liver after partial hepatectomy (mean ± SD)

Time	Weight (gm) Mean + S.D	Water content Mean + S.D	Lipid content Mean + S.D
	4.094± 0.212		
0 day	1.271± 0.182***	70.224± 0.258	2.503± 0.252
1 day	1.842± 0.189***	73.058± 0.878**	4.830± 0.732***
2 day	2.547± 0.297**	75.655± 1.660***	4.439± 0.627***
3 day	3.94± $0.2398^{N.S}$	73.445± 0.880**	3.157± 0.325**
7 day	3.94± $0.2398^{N.S}$	73.214± 0.869**	2.63± $0.278^{N.S}$
14 day	4.009± $0.260^{N.S}$	71.755± 0.548*	2.393± $0.346^{N.S}$
28 day	4.019± $0.183^{N.S}$	70.738± $0.483^{N.S}$	2.439± $0.169^{N.S}$

*Data are expressed as mean ± SD. *Significant at P≤ 0.05;** Significant at P ≤ 0.01; *** Significant at P ≤ 0.001. Initial liver weight= 4.094±0.212gm.*

The decreased rate of hepatic protein synthesis observed in hepatectomized rats, may be attributed to that the hypercatabolism induced by hepatectomy is significantly greater [17]. However, regeneration of liver protein mass can be achieved by 2 mechanisms, either by increased protein synthesis or by decreased protein breakdown [18].

Normally, livers of mature rodents show little mitotic activity, but it has long been known that mitosis can be stimulated surgically by performing a PH or by injecting a hepatocellular toxic agent [23]. The present study have shown that the frequencies of cells with Mn in day 0 (control) are low, however, the frequencies of cells with Mn can be increased after hepatectomy and that confirming the findings of [23].

Transaminases (ALT, AST, ALP, and BR) are the most commonly used clinical indicators for the evaluation of liver function [19]. In our study the level of these enzymes was decreased significantly after PH by days 1- 7, then increased significantly for restoring its normal level by day 14. The alteration of these enzymes following liver injury may be associated with trauma and changes in intraoperative blood flow and have an important role in the evaluation of liver function following surgery [20].

Table 2. Effect of time (days) on Micronucleus, DNA fragmentation and hemoglobin level after partial hepatectomy in rats

Time	Cell	MN	% DNA Fragmentation	Hemoglobin
0 day	2000	2.6 ±	1.944±	14.36±
		1.2	0.130	1.270
1 day	2000	22.8±	5.762±	5.2±
		3.429***	0.868***	1.034***
2 day	2000	15.5±	2.979±	8.53±
		2.872***	0.232***	1.068***
3 day	2000	11.4±	2.559±	9.65±
		2.835***	0.329**	1.040***
7 day	2000	9±	2.133±	11.24±
		2.449***	0.230 N.S	0.795**
14 day	2000	7±	2.028±	12.44±
		2.50**	0.281 N.S	1.46**
28 day	2000	3±	1.912±	14.26±
		1.414 N.S	0.244 N.S	1.397 N.S

*MN: micronucleus; Data are expressed as mean ± SD. *Significant at P≤ 0.05; ** Significant at P ≤ 0.01; *** Significant at P ≤ 0.001.*

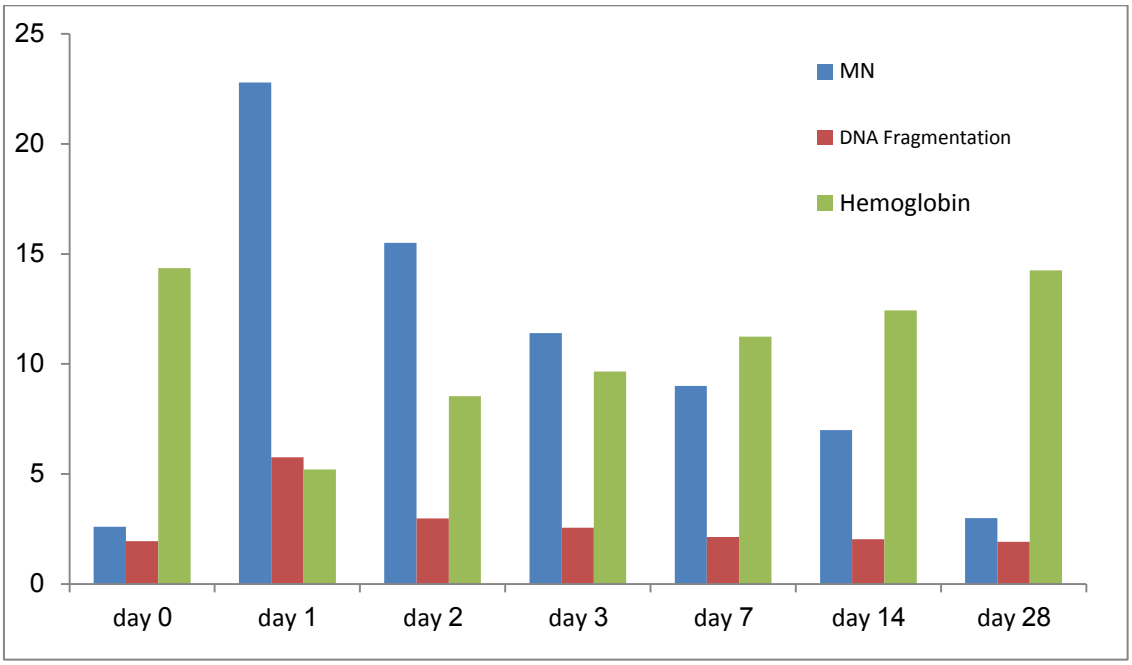

Fig. 2. Effect of time (days) on Micronucleus, DNA fragmentation and hemoglobin level after partial hepatectomy in rats

Table 3. Effect of time (days) on liver function of rats after partial hepatectomy

Time	AST (u/L)	ALT (u/L)	ALP (u/L)	BR (umol/L)	Protein (g/dL)
0 day	50.646± 1.050	52.158± 1.325	288.947± 18.978	0.398± 0.20	13.181± 0.554
1 day	21.48± 1.163***	21.951± 1.529***	149.948± 14.396***	0.127± 0.022***	7.4250± 1.183***
2 day	26.305± 1.451***	28.133± 2.150***	186.628± 17.657***	0.213± 0.018***	9.188± 0.999***
3 day	36.224± 2.7113**	40.596± 1.693**	212.947± 10.198**	0.234± 0.021***	11.782± 0.703**
7 day	42.934± 1.603**	46.196± 1.502*	243.555± 11.865**	0.289± 0.019**	12.516± 0.661*
14 day	49.694± 1.247 N.S	51.561± 1.360 N.S	275.531± 15.764 N.S	0.369± 0.030 N.S	12.608± 0.693 N.S
28 day	51.057± 1.486 N.S	51.94± 1.424 N.S	287.089± 20.137 N.S	0.390± 0.026 N.S	13.202± 0.736 N.S

*AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase;BR: bilirubin. Data are expressed as mean ± SD.*Significant at P≤ 0.05; ** Significant at P ≤ 0.01; *** Significant at P ≤ 0.001.*

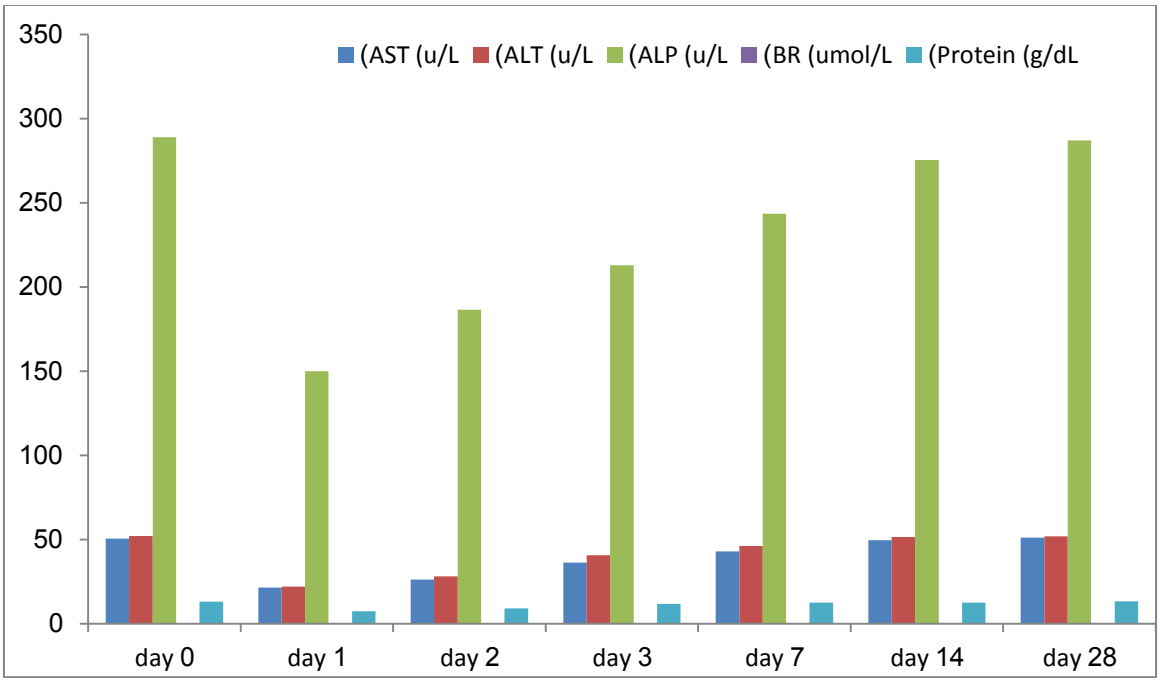

Fig. 3. Effect of time (days) on liver function of rats after partial hepatectomy

5. CONCLUSION

The findings from this study in a male rat model of liver regeneration following partial liver hepatectomy, indicated that 70% PH in rats induces a rapid regenerative response. In our study rats lost liver weight until POD 2, after which there was a steep increase in weight reaching a plateau slightly near the preoperative weight on POD 3. We evaluated liver function in the postoperative period by measuring transaminase enzymes (ALT, AST, ALP and BR and protein levels. We found low levels of these enzymes on PODs 1-7, consistent with resection associated liver injury, after which levels raise to normal concentration on day 14. However, lipid content was increased significantly in operated animals but restored to normal level by day 7. Water content was similarly increased significantly in regenerating liver tissue and restored the normal level by day 28 following operation. In addition, hemoglobin was decreased and restored to normal by day 28 and the percentage of DNA fragmentation and micronucleus frequency were increased significantly compared to control (day 0) and restored to normal on day 7 and

28 for DNA and Mn, respectively. Our results might be of clinical relevance for human medicine.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data and materials are available.

CONSENT

All authors declare that this manuscript is consent for publication.

ETHICAL APPROVAL

This work was approved by the Medical Research Ethical Committee (MREC) of the National Research Center, Dokki, Giza, Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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