

Effect of the contraceptives pills on liver function in mice infected with visceral Leishmaniasis

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ABSTRACT

To know the effect of the contraceptive pills on liver enzymes GOT, GPT in infected mice with visceral Leishmaniasis, ingested orally with the pills with a dose 0.012 mg/day and comparisons with pentostam drug 0.4 mg/day treated with 21 days. The results showed that the pills led to a significant increase in GOT and GPT in the third weeks, compared to the pentostam group. It could be concluded that the contraceptive pills had a clear role in increasing the severity of the parasite infection in the laboratory animals by the observed increase in these physiological indicators.

Keywords: leishmania donovani, the contraceptive pills, liver enzymes GOT, GPT.

INTRODUCTION

Visceral leishmaniasis (VL) can cause death in 100% of infected patients if left untreated¹. In 2015, VL represents a major public health problem worldwide and is classified as one of the neglected tropical diseases². VL, the cause is protozoa of the genus *Leishmania donovani* complex, its origin from animal infections transmitted by a vector-borne³. kala-azar is characterized by substantial weight loss, irregular bouts of fever, and swelling of the liver and spleen that leads to anemia⁴. The cause of clinical manifestations of VL has been attributed to the invasion of the reticuloendothelial cells of the spleen, liver and bone marrow and the subsequent proliferation of amastigotes inside cells⁵. Oral contraceptives (OC) are daily hormonal pills that help change the way the body works effectively⁶.

They change the lining of the uterus that become hostile to fertilized ova, change the type of mucus secretion in the cervix to prevent sperm migration through cervical canal⁷. There are two types of oral contraceptives, combined oral contraceptive tablets and non-combined ones that contain only progestin while the combined oral contraceptives contain artificial or synthetic forms of progestin and estrogen hormones⁸. Estrogen and progesterone is steroid hormones, these hormones are lipophilic molecules, used as chemical messages by organisms⁹. Recent experimental evidence indicates that parasites can exploit the host by using the micro-hormonal environment within it to favor their formation, growth and reproduction¹⁰. likewise when assessing the effects of progesterone on the morphology, maturation, and behavior of *Haemonchus contortus* larvae in vitro and directly, whereas adding different concentrations of progesterone to the larval cultures significantly inhibited molting and increased larval motility compared to non-stimulated larvae¹¹.

MATERIALS AND METHODS

Parasite strain and culture: *Leishmania donovani* was cultured and maintained by serial passage in NNN media each 8 days and incubated at 27 C°¹².

Animal grouping: Seventy-two mice were infected with 1x10⁷ parasite/mL of *Leishmania donovani* promastigotes

injected intraperitoneal and the remaining number of uninfected mice was considered a control group¹³.

Group1: ingested orally with normal saline (non infected).

Group2: ingested orally with normal saline and considered as positive control (infected)

Group3: ingested orally with contraceptives pills.

Group4: injected by Pentostam drug intramuscularly and considered as treated group.

Group5: ingested orally by contraceptives and injected by Pentostam drug intramuscularly.

Blood collection: After the 7th, 14th, 21th days, 2 ml of blood was collected from mice from an ocular vein and placed in a gel tube, after that, left at room temperature for 35 minutes. Centrifugation was performed and the serum obtained and the level of each GOT and GPT were measured.

Measurement of liver enzymes: The principle of work of both Glutamic- oxaloacetic transaminase (GOT) and Glutamate – pyruvic transaminase (GPT) were done according to the instructions of manufacture by Spin 200. The same company manufactures the kit uses in this device and the method to work is the open system.

Statistical analysis: the statistical analysis was carried out by SPSS (v 20). Data expressed as mean ± SD.

RESULTS

Measurement of liver enzymes: the results in this study as shown in table 1, 2 in demonstrating that there were significant changes in the level as follows:

The GOT level, increase in the positive control (G2) compared with the negative control (G1) after 7th day, were 31.17,91.83 U / L respectively, in the other groups (G3, G4, G5) there was a significant increase in the GOT level were 116.83,106.83and 83.33 U / L respectively, in G2, G3 the GOT level continued to increase until the 21th day was 215.00, 260.67 U / L, while the level of GOT decreased in G4 and G5 28.33,30.50 U / L, respectively, and these results showed whether increased or decreased the presence of significant differences between.

The GPT level, increase in the positive control (G2) compared with the negative control (G1) after 7th day was 20.67, 171.83 U / L respectively, the other groups G3,

G4, G5 there was a significant increase in the GPT level were 58.17,173.83,137.83 U / L respectively, in G2and G3 the GPT level continued to increase until the 21th day was (404.67, 435.17) U / L, while the level of GPT decreased in

G4 and G5 (39.17, 37.83) U / L respectively, and these results showed whether increased or decreased the presence of significant differences between.

Table 1: The serum level of GOT U/L in the study groups

GOT U/L	7 days		14 days		21 days		P value
	Mean	±SE	Mean	±SE	Mean	±SE	
Negative control(G1)	31.17	3.15	31.17	3.15	31.17	3.15	-----
Positive control(G2)	A 91.83	1.99	B 195.83	1.58	B 215.00	3.54	0.001
contraceptives pills(G3)	B 116.83	3.82	B 207.00	2.16	C 260.67	13.16	0.001
Pentostam drug(G4)	B 106.83	1.78	A 61.83	2.04	A 28.33	1.45	0.001
contraceptives pills + pentostam(G5)	A 83.33	1.98	A 54.17	1.40	A 30.50	1.31	0.001
P value	0.001		0.001		0.0001		
LSD	14.78		19.31		16.09		

LSD test was used to calculate the significant differences between tested mean, the letters (A,B,C and D for column)represented the levels of significant ,highly significant start from the letter (A) and decreasing with the last one. Similar letters mean there are no significant differences between tested mean.

Table 2: The serum level of GPT U/L in the study groups

GPT U/L	7 days		14 days		21 days		P value
	Mean	±SE	Mean	±SE	Mean	±SE	
Negative control(G1)	20.67	0.88	20.67	0.88	20.67	0.88	NS
Positive control(G2)	C 171.83	1.87	C 314.50	9.96	B 04.67	6.18	0.01
contraceptives pills(G3)	A 58.17	1.70	B 175.17	5.79	C 435.17	15.09	0.01
Pentostam drug(G4)	C 173.83	2.04	A 89.50	2.25	A 39.17	1.35	0.01
contraceptives pills + pentostam(G5)	B 137.83	1.35	A 82.17	1.72	A 37.83	1.87	0.01
P value	0.01		0.001		0.001		
LSD	17.6		22.04		19.32		

LSD test was used to calculate the significant differences between tested mean, the letters (A,B,C and D for column)represented the levels of significant ,highly significant start from the letter (A) and decreasing with the last one. Similar letters mean there are no significant differences between tested mean.

DISCUSSION

Murray [2001]¹⁴ explained that the cause of the rise was *Leishmania donovani*, which destroyed the liver and spleen cells, these injured cells released it into the circulation. This increase reflects the pathological picture of non-specific granulomatous formation in the patients' liver. Kaye *et al* [2004]¹⁵ showed that the parasite was ultimately eliminated from the liver and that hepatic resistance to infection results from a coordinated host response that includes a wide range of effector and regulatory pathways targeted within specific tissue structures called granulomas. The persistence of the parasite is accompanied by failure of granuloma formation and a variety of pathological changes, including an enlarged spleen, disruption of the microstructure of lymph tissue, and enhanced hematopoietic activity. Tesfanchal *et al* [2020]¹⁶ showed that the change in liver function may be due to an enlarged liver and the formation of an immune complex in VL patients. It has also been recorded that the production of reactive oxygen species from activated macrophages has contributed in liver function changes. Rodrigues *et al* [2016]¹⁷ reported that the Th1 response in turn against *leishmania donovani* is initiated by IL-12 secreted from the dendritic cells , IL-12 is an essential cytokine in the development of protective immunity to L. donovani, since blocking of IL-12 reduced both the IFN γ production and granuloma formation in the liver of infected mice, this blocking leads to an increase in the level of both GOT and GPT.

Also Mathur *et al* [2008]¹⁸ demonstrated that there was an increase in the level of GOT and GPT in confirmed cases of visceral leishmaniasis. Likewise Ferreira *et al* [2020]¹⁹ mentioned the disturbance of host metabolic pathways by *Leishmania donovani* has crucial consequences for the activation status of immune cells and the outcome of infection, so GOT level rises.

Furthermore, Taher *et al* [2015]²⁰ showed serum GPT and GOT activity was significantly increased in all cases of kala-azar studied in comparison to the normal controls, their high levels were indicative of liver damage. The GPT was increased when infected with *Leishmania donovani* because it attacks the visceral organs, especially the liver, and thus the high GPT level is a sign of liver damage, the progressive anemia that occurs as a result of the presence of the parasite leads to the bone marrow not producing enough blood, and this gives instructions to the liver to produce red blood cells, and because there is an abnormal increase in production, it leads to gradual damage to the liver due to the presence of a parasite feeding on it and thus it was observed that GPT level continues to rise [Prajapati *et al.*, 2016]²¹.

This is confirmed in a study of patients with visceral leishmaniasis; the anemia is one of the most remarkable manifestations of VL, it occurs when the spleen becomes palpable and progresses with its gradual increase; the cause of this type of anemia, is partly caused by red blood cell destruction, multifactorial (splenic phagocytosis of opsonized erythrocytes, hemodilution, and increased destruction of normal red blood cells by hypersplenism), therefore, the liver in conjunction with the spleen, when

infection occurs, the destruction of red blood cells leads to an increase in the level of both GOT and GPT, in addition to this, bone marrow failure in the regeneration of red blood cells, which are removed from the circulation, this is attributed to poor nutrition and the spread of infection [Pedrosa, 2017]²².

CONCLUSION

Ingestion the infected mice with contraceptive pill leads to increasing the severity of infection. Also contraceptive pills led to increase the level of liver enzymes GOT and GPT *in vivo*.

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