Plant Archives Volume 20 No. 2, 2020 pp. 7213-7218 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

HEPATIC EFFECTS OF P-GLYCOPROTEIN INHIBITOR (CAPTOPRIL) AND P-GLYCOPROTEIN INDUCER (SPIRONOLACTONE) WITH THEIR SUBSTRATE (LOVASTATIN) IN MALE RATS

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Abstract

The present study was performed to estimate the effect of P-glycoprotein inhibitor (captopril) and inducer (spironolactone) with their common substrate (lovastatin) on liver functions in rats after two periods of administration at 45 days and 90 days with alone and combined therapeutic doses. Thirty rats were used to perform the liver enzymes such as (serum alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP)) and lipid profile in blood serum. The animals of study divided equally into six groups according to daily dosing regimen as following G1- control group administered distilled water orally, G2 administered orally captopril 0.7 mg/kg b.w., G3- administered orally spironolactone 1.4 mg/kg b.w, G4- administered orally lovastatin 0.57 mg/kg b.w, G5 -administered orally spironolactone 1.4 mg/kg b.w. and lovastatin 0.57 mg/kg b.w, G6- administered orally captopril 0.7 mg/kg b.w and lovastatin 0.57 mg/kg b.w. The results of experiment showed that there is a significant increase ($p<0.05$) in serum alanine amino transferase (ALT) between groups at day 45 and day 90 of treatment also there were significant differences between different drugs alone or combined, since combined groups showed significant increase in both periods compared with other groups. Also the results indicated elevation in serum aspartate amino transferase (AST) during the experiment in all treated groups. As well as the results of effects of different drugs alone and in combination on alkaline phosphatase (ALP) revealed a significant increase ($p<0.05$) in serum ALP in CP+LV group due to increase effect of increase its accumulation at both times in comparison with controls groups. The results of lipid profiles revealed that some significant differences in treated groups at 45 days and 90 days, these significant differences were in LV group showed decrease in total cholesterol and its derivatives (TAG, LDL, HDL and VLDL) after 90 days in comparison with control group. Also in combined administration groups especially CP+LV group showed decrease in total cholesterol and improve the high density lipoprotein.

Key words: Hepatic effects, ALP, AST, inhibitors.

Introduction

Adenosine triphosphate (ATP)-binding cassette (ABC) transporters are highly expressed in tumor cells, as well as in organs involved in absorption and secretion processes, mediating the ATP-dependent efflux of compounds, both endogenous substances and xenobiotics, including drugs. Their expression and activity levels are modulated by the presence of inhibitors, inducers and/or activators (Gameiro *et al.,* 2017). The first known human ABC transporter was Pglycoprotein (P-gp), which confers multidrug resistance (MDR) to anticancer drugs (Litman *et al*., 2001).

P-glycoprotein (P-gp) is an ATP-dependent efflux pump encoded by the MDR1 gene in humans, known to mediate multidrug resistance of neoplastic cells to cancer therapy (Silva *et al.,* 2015).

P-glycoprotein (P-gp) is physiologically expressed at the bile canalicular membrane of the liver, where it participates in the biliary excretion of various drugs (Yu *et al.,* 2010). P-gp is exposed to drug molecules after being cellularly uptaken, distributed, and metabolized in both the liver and the kidney (Srinivas, 2008). P glycoprotein consider as phase III transporters in metabolism, which are present in abundance either at the basal uninduced level and/or inducible at elevated level after xenobiotics exposure (Rushmore and Kong,

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2002; Wang and LeCluyse, 2003). Inhibition or induction of drug transporters by coadministered drugs can alter pharmacokinetics and pharmacodynamics of the victim drugs (König *et al*., 2013). Hyperlipidemia is the term refers to increased concentrations of lipids (triglycerides, cholesterol, or both) in the blood stream, increased blood concentrations of triglycerides referred to as hypertriglyceridemia, while increased blood concentrations of cholesterol are referred to as hypercholesterolemia (Xu *et al*., 2014). Captopril is an angiotensin-converting enzyme (ACE) inhibitor which was initially approved to treat high blood pressure and can be used alone or in combination with other antihypertensive drugs (Miguel-Carrasco *et al*., 2010). Drug- drug interaction between captopril and statins when co-administered can also reduce their efficacy and changing their availabilities inside body (Tabassum *et al*., 2016). Statins are a widely prescribed class of drugs to decrease cholesterol. Their mechanism of action is primarily via inhibition of HMG-CoA (hydroxymethylglutaryl-coenzyme A) reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway (Hess *et al*., 2018). Benefits of statin Therapy to support reducing LDL (low-density lipoprotein cholesterol) and enhance the HDL(high low density lipoprotein cholesterol) to reduce atherosclerotic cardiovascular disease (CVD) (Ference *et al*., 2017). Many side effects of statin drugs, which can be more serious, include new-onset type 2 diabetes mellitus, hepatotoxicity, renal toxicity, and other conditions (Bitzur *et al*., 2013). Spironolactone is an aldosterone antagonist and a potassium-sparing diuretic used for treatment of hyperaldosteronism and edematous states including congestive heart failure and liver cirrhosis (Batterink *et al*., 2010). SN causes induction of P glycoprotein expression with potential impact on intestinal absorption of substrates with therapeutic application (Ghanem *et al*., 2006).

Materials and methods

A thirty adult Albino male rats were obtained from animal house of college of veterinary medicine\ University of Baghdad, They aged over 3 months and weighed 200-250 g., used to perform different studies. They were fed standard pellet diet and drink tap water. The animals were left in special cages with optimal conditions two weeks for adaptation in the animal house of veterinary medicine, maintained with standard condition at 10/14 hour light - dark cycle, (20-25^{o}C) in an air conditioned room. The bed were mulch that changed twice per week. These studies were performed under the rules of ethics for management laboratory animals submitted by University of Baghdad and done under supervision of sided committee in the College of Veterinary Medicine. In which animals divided equally into six groups according to daily dosing regimen as following G1- control group administered distilled water orally, G2 administered orally captopril 0.7 mg/kg b.w., G3- administered orally spironolactone 1.4 mg/kg b.w, G4- administered orally lovastatin 0.57 mg/kg b.w, G5 -administered orally spironolactone1.4 mg/kg b.w and lovastatin 0.57 mg/kg.b.w, G6- administered orally captopril 0.7 mg/kg.b.w and lovastatin 0.57 mg/kg.b.w. The drugs of this study were purchased from the pharmacy.

The period of administration was 90 days, since the blood withdrawn from 5 rats for each group at 45 days and then at 90 days in experiment end. The blood was collected in gel tube at room temperature and then the serum was separated by centrifugation at 3000 rpm for 10 minutes and then kept frozen at –20°C to be analyzed thereafter. The serum obtained from the blood samples from all participants in this study was used to estimate.

1. Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were determined using kit supplied by BioLabo Company France.

2. Serum alkaline phosphatase activity (ALP) was estimated using kit supplied by BioLabo, France.

3. Serum bilirubin concentration was measured colorimetrically using kit supplied by Bio Labo, France.

4. Lipid profile in serum was measured by using kit supplied by Bio Labo Company, France.

Statistical analysis

Statistical analysis of data was performed on the basis of One way and Two way analysis of Variance (ANOVA) using a significant level of ($p>0.05$) .Specific groups differences were determined using Least Significant Differences (LSD) as described by (Snedecor and Cochran, 1980).

Results and Discussion

Serum liver enzymes

1. Alanine amino transferase (ALT) and Aspartate amino transferase (AST):

The present study illustrated in table 1 showed a significant increase ($p<0.05$) in serum alanine amino transferase (ALT) between groups at day 45 and day 90 of treatment also there were significant differences between different drugs alone or combined, since combined groups (35.5 U/l, 36 U/l) showed significant increase in both periods with alone drugs recording in CP

Table 1: Effect of captopril, spironolactone and lovastatin alone and in combination on alanine amino transferase (ALT) and on aspartate amino transferase (AST) in male rats.

Groups	ALTTime		ASTTime	
	45 day	90 day	45 day	90 day
Control	25 ± 0.57 D a	24 ± 0.40 D a	53 ± 0.40 E a	52.5 ± 0.28 F a
SN	30 ± 0.35 B b	36 ± 0.40 B a	69 ± 0.40 D b	84.3 ± 0.33 D a
CP	30.5 ± 0.35 B b	35.3 ± 0.47 B a	69.3 ± 0.50 D b	73 ± 0.35 E a
LV	28.5 ± 0.28 C b	32.3 ± 0.46 C a	109 ± 0.40 A b	113 ± 0.35 A a
SN + LV	35.5 ± 0.35 A b	41.3 ± 0.43 A a	70 ± 0.40 C b	88.6 ± 0.30 C a
CP + LV	36 ± 0.40 A b	40.5 ± 0.64 A a	74.5 ± 0.28 B b	107 ± 0.36 B a
LSD0.05	1.265		1.072	

*Capital litters refer to vertical statistical comparison whereas the small letters refers to the horizontal statistical comparison.

* The values with similar letters are non-significantly difference ($p<0.05$).7

and SN (30.5, 30) U/l except LV group (28.5 U/l) showed significant decrease in ALT compared with other groups. Also this table revealed a significant increase ($p<0.05$) in serum aspartate amino transferase (AST) in all groups at period day 45 and at day 90 and between groups compared with controls groups, while groups of CP and SN showed no significant at day 45 but the same groups denote significant difference at day 90 (73, 84.3) U/l respectively.

Alkaline phosphatase (ALP):

The results of effects of different drugs alone and in combination on alkaline phosphatase (ALP) revealed a significant increase ($p<0.05$) in serum ALP in all groups at both times and between groups in comparison with controls groups.

Table 2: Effect of captopril, spironolactone and lovastatin alone and in combination on alkaline phosphatase (ALP) in male rats.

Groups	Time	
	45 day	90 day
Control	70 ± 0.35 F a	69.3 ± 0.34 F a
SN	84.3 ± 0.33 E b	109.5 ± 0.35 E a
CP	98 ± 0.44 C b	155.3 ± 0.33 C a
LV	147 ± 0.35 A b	272 ± 0.35 A a
SN + LV	88.3 ± 0.37 D b	123.5 ± 0.28 D a
CP + LV	102 ± 0.40 B b	260.3 ± 0.45 B a
LSD0.05	1.049	

3. Serum bilirubin (total and indirect):

In this study, the results of total and indirect serum bilirubin showed no significant differences in all groups between both periods and between the treated groups in comparison with the controls groups as described in following table 3.

Serum lipids profile

In our present study we tested the three drugs (CP, SN, LV) alone and in combination (CP + LV, SN + LV) to get the effects of those drugs on profile of lipids in blood serum of experimental animals. The results showed a significant decrease ($p<0.05$) in serum of cholesterol, serum triglyceride, low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) of treated groups at day 45 and at day 90 and between all treated groups in comparison with control groups, while the results of serum of high density lipoprotein (HDL) cholesterol revealed a significant increase ($p<0.05$) in all treated groups at day 45 and day 90 compared with controls groups as described in the following tables 4, 5, 6, 7 and 8.

Understanding of the pathophysiological functions of transporters and their role in drug disposition, efficacy and toxicity may help in the end to improve therapeutic efficacy of new drugs and to allow safer drug treatment. Moreover, it could open new treatment strategies for various diseases, by optimizing drug delivery to intracellular targets, selected organs and to sanctuary sites (Marchetti *et al.,* 2013).

Elevated liver enzymes are a common scenario encountered in clinical practice. So evaluation of this a problem presenting with no symptoms can be challenging.

Table 3: Effect of captopril, spironolactone and lovastatin alone and in combination on total and indirect bilirubin (mg/dl) in male rats.

Groups	Total bilirubin Time		Indirect bilirubin Time	
	45 day	90 day	45 day	90 day
Control	0.202 ± 0.002 A a	0.211 ± 0.003 A a	0.112 ± 0.001 A a	0.115 ± 0.001 A a
SN	0.204 ± 0.0006 A a	0.205 ± 0.004 A a	0.113 ± 0.002 A a	0.122 ± 0.001 A a
CP	0.205 ± 0.002 A a	0.208 ± 0.004 A a	0.115 ± 0.003 A a	0.119 ± 0.004 A a
LV	0.205 ± 0.004 A a	0.207 ± 0.006 A a	0.116 ± 0.003 A a	0.119 ± 0.003 A a
SN + LV	0.203 ± 0.0004 A a	0.204 ± 0.003 A a	0.123 ± 0.004 A a	0.129 ± 0.002 A a
CP + LV	0.206 ± 0.003 A a	0.204 ± 0.002 A a	0.114 ± 0.002 A a	0.125 ± 0.006 A a
LSD0.05	0.0071		0.0112	

In general, the effects in liver enzymes due to the drugs of this study were mild effects because the doses were in normal range and low toxic effect (Malakouti *et al.*, 2017).

Our data revealed that there is a significant increase in liver enzymes ALT, AST and ALP in all treated groups at day 45 with more increase at day 90 in comparison with control group especially in LV alone group and

CP+LV combined group which could be attributed to their accumulative effect due to their chronic daily dosing causing accumulation of LV and CP which increase their pharmacological and/or toxic effect on the liver. This was concluded from our data of chronicity index (comparison of acute LD_{50} and LD_{50} after chronic exposure) which indicate for sure the accumulative nature of both drugs (Aboktifa and Abbas, 2020).

Also we must take in consideration that captopril is P-gp inhibitor that mean it might change the kinetic of the same drug or other drug (LV substrate) gives in combination in such way that increase their absorption and decrease their hepatic excretion that caused increase in their body burden with increase in their efficacy or even toxic effect.

The interesting results of chronicity index study in press were the higher result (1.7 and 3.6) for alone and combined captopril with lovastatin which made us conclude that captopril possibly might act as P-gp inhibitor/substrate, the matter that explain the competition with lovastatin for their kinetics and toxic effect. These are affecting the level of hepatic enzymes at both experimental periods.

These results correspond to a noticed by Forrester J.S. and Libby P. 2007 and Argo *et al.,* 2008 who confirmed treatment by statins leading to elevations in liver enzymes that often occurred in 12 weeks of therapy because association of statin treatment with a broad spectrum of adverse effects of hepatocyte. Although the basic mechanism remains unclear, it may result from changes in the components of lipid of the hepatocyte membrane, leads to an increase in its permeability of liver enzymes "leakage". This is occurred by the elevations in aminotransferase levels due to statins using, as well as with other effective lipid-lowering drugs (Bhardwaj and Chalasani (2007); Chang and Schiano (2007). Significant increase in the level of serum enzymes of liver (ALT, AST and ALP) in all groups were noticed after 3 months of treatment period in comparison with the control. This finding is similar to that of Cheng and Harris, 2004 and Witwit, 2008 Who found that many medication cause

Table 4: Effect of captopril, spironolactone and lovastatin alone and in combination on cholesterol (mg/dl) in male rats.

Groups	Time	
	45 day	90 day
Control	89 ± 0.20 A a	87 ± 0.40 A b
SN	77.5 ± 0.38 D a	70.3 ± 0.31 C b
CP	81.6 ± 0.51 C a	74 ± 0.36 B b
LV	61.3 ± 0.16 F a	51.2 ± 0.14 F b
SN+LV	70.3 ± 0.31 E a	56.3 ± 0.27 E b
CP+LV	84 ± 0.40 B a	58.5 ± 0.29 D b
LSD0.05	0.954	

Table 5: Effect of captopril, spironolactone and lovastatin alone and in combination on triglyceride (mg/dl) in male rats.

Groups	Time	
	45 day	90 day
Control	91 ± 0.45 A a	90 ± 0.17 A b
SN	84 ± 0.17 B a	55.6 ± 0.14 B b
CP	59.5 ± 0.40 D a	46.6 ± 0.27 C b
LV	39.5 ± 0.40 F a	37.6 ± 0.27 D b
SN+LV	50 ± 0.40 E a	30 ± 0.40 F b
CP+LV	73.5 ± 0.22 C a	35 ± 0.40 E b
LSD0.05	0.923	

Table 6: Effect of captopril, spironolactone and lovastatin alone and in combination on low density lipoprotein (LDL) cholesterol (mg/dl) in male rats.

Groups	Time	
	45 day	90 day
Control	40 ± 0.35 A a	39.35 ± 0.42 A a
SN	18.5 ± 0.22 D a	15 ± 0.21 D b
CP	31.6 ± 0.31 B a	21 ± 0.17 B b
LV	14.5 ± 0.21 F a	12 ± 0.35 E b
SN+LV	17 ± 0.20 E a	15 ± 0.49 D b
CP+LV	30.6 ± 0.29 C a	19 ± 0.25 C b
LSD0.05	0.886	

Table 7: Effect of captopril, spironolactone and lovastatin alone and in combination on very low density lipoprotein (VLDL) (mg/dl) in male rats.

Groups	Time	
	45 day	90 day
Control	20 ± 0.40 A a	18 ± 0.40 A b
SN	16.25 ± 0.32 B a	11.3 ± 0.36 B b
CP	8 ± 0.49 E a	6.5 ± 0.49 D a
LV	6 ± 0.28 F a	4.3 ± 0.46 F b
SN+LV	10 ± 0.42 D a	5.77 ± 0.31 E b
CP+LV	12.5 ± 0.41 C a	7 ± 0.42 C b
LSD0.05	1.170	

Table 8: Effect of captopril, spironolactone and lovastatin alone and in combination on high density lipoprotein (HDL) (mg/dl) in male rats.

Groups	Time	
	45 day	90 day
Control	29 ± 0.26 D a	30.2 ± 0.43 E a
SN	43 ± 0.40 A a	44 ± 0.40 C a
CP	41.5 ± 0.34 B b	46 ± 0.55 B a
LV	40.9 ± 0.57 B b	44 ± 0.40 C a
SN+LV	39 ± 0.45 C b	37.6 ± 0.35 D b
CP+LV	43 ± 0.32 A a	50 ± 0.42 A b
LSD0.05	1.211	

increases in liver enzymes.

Bilirubin is a neurotoxin in neonates; however, current evidence has confirmed that an increase in serum bilirubin concentration in physiological ranges is associated with both chronic kidney disease (CKD) and cardiovascular disease (CVD) in adults (Tsai and Tarng, 2019). P-glycoprotein in blood brain barrier may play a role in limiting the entry of bilirubin into the brain (Tsai *et al.,* 2002). Our data revealed that no significance differences in and between all groups of experiment for total and indirect serum bilirubin due to therapeutic effects of these drugs but not toxicity effects, as well as treatment by this drugs depending on the duration of dosage and doses to cause hyperbilirubinemia.

The lipids can be classified as Total Cholesterol (TC) and its derivatives such as; Triglycerides (TAG), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Very Low Density Lipoprotein (VLDL) cholesterol (Onwe *et al*., 2015). In our study the results revealed that some significant differences in treated groups at 45 days and 90 days, these significant differences were in LV group showed decrease in total cholesterol and its derivatives (TAG, LDL, HDL and VLDL) after 90 days of administration as explained by Castro *et al.,* 2017. Also in combined administration groups especially CP+LV group that lead to increase in effects of two drugs in lowering total cholesterol because the diuretics effects of captopril and spironolactone as showed by Mahmoudabady *et al.,* 2015 and Polyzos *et al.,* 2011 who pointed that these drugs causes decrease in total cholesterol, triglyceride and enhance high density lipoprotein.

In conclusion from our data it become clear that P-gp inhibitor increase the efficacy and hepatic effect of it's substrate by increase its' burden and their level in blood and liver, while spironolactone not showed such clear effect on liver when combined with lovastatin possibly because lovastatin effect in liver overcome or reduce the spironolactone effect.

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