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Determination of Buparvaquone Residues in Rabbit Tissues Using Hplc and its Effect on Different Liver and Kidney Functions

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Abstract | The aim of this study was to determine residues of buparvaquone (BPQ) in different rabbit tissues and evaluate its withdrawal time in healthy rabbits after single intramuscular infusion. Twenty-four healthy rabbits were classified into two equal groups (n=12); the first group were injected with distilled water and considered as control. The other group was injected with BPQ 2.5 mg/kg intramuscularly. The samples were harvested at 7th, 14th, 30th and 42th day post-injection. Tissue (liver, kidney, thigh muscle) and serum samples were kept frozen until were analyzed. BPQ residues were utilized with high performance liquid chromatographic method. Renal and hepatic function markers were evaluated in response to infusion of BPQ. The results revealed presence of drug residues in all tissues with the highest level within liver, kidney and muscle in comparison to the control animals. Residues completely disappeared from the examined tissues at 42th-day post-infusion except liver. In conclusion, rabbit muscles and kidneys, but not liver, might be safe for the human consumption at 42-day post-BPQ administration.

Keywords | HPLC, Buparvaquone, Residues, Liver functions, Kidney functions, Rabbits.

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INTRODUCTION

Buparvaquone (BPQ) is a second-generation hydroxy-naphthoquinone anti-theilarial medication identified to parvaquone and atovaquone (Müller et al., 2015). It is an effective medication for curing and prevention of all types of theileriosis that can be administrated during incubation period or after appearance of disease symptoms (Hashemi-Fesharki, 1991). Buparvaquone was also successfully used for treatment of leishmaniasis, which is a very widely distributed disease all over the world caused by protozoan parasites of the Leishmania, which cause a scope of sicknesses in people. Owing to its antileshmanial activity against *Leishmania donovani* that found in more than 80 countries in Asia and Africa, BPQ administrated subcutaneously in corn oil by a dose of 100 mg/kg/day for 5 days (Garnier et al., 2007). Additionally, BPQ is also used in treatment of equine piroplasmosis, which is widely distributed all over the world. It was found to be transplacental transmitted from infected mares to foals (Chabra et al., 2012). Treatment with imidocarb dipropionate at 4 mg/kg intramuscularly removes *T. equi* from horses (Berlin et al., 2010), but causes deaths in donkies (Soulsby, 1982). The clearance of disease was reported after injection of BPQ in a dose of 2.5 mg/kg. The efficiency of BPQ) Buparvon (in treatment of theileriosis in cattle was evaluated, and was injected intramuscularly in the cattle with theileriosis using an only dose of 2.5mg/kg. It proved that single deep intramuscular injection was effective (Pasa, 2008).

Since there are several uses of BPQ in animals worldwide, this study was therefore performed to determine its with-

drawal time from the different tissues of rabbit as well as evaluate its impact on renal and hepatic functions (Garba et al., 2015).

MATERIAL AND METHODS

Experimental animals

A total of twenty-four healthy rabbits [Baladi breed, both sexes, and weighing 2-2.5 kg] were involved in this investigation. The rabbits were permitted for adjustment for 7 days with free admittance to water and pelleted diet. There were no significant abnormalities clinically noted on those rabbits. This study was conducted according to the institutional guidelines for regulation of the care and use of animals.

Experimental design

Rabbits were arbitrarily isolated into two groups with twelve rabbits for each. Group-1 employed as a control and was treated with distilled water. Group-2 was intramuscularly infused on the thigh muscle with BPQ at the dosage of 2.5-mg/kg body weight.

In both groups, serum samples were gathered, and then the animals were sacrificed at 7, 14, 30 and 42 day time points post-infusion (n=3 at each time point). After scarification, livers, thigh muscles, and kidneys were also collected from each animal and stored at -80^{0}C until were analyzed.

BPQ Residues Extraction

BPQ residues were extracted from tissues with acetonitrile/acetone and cleaned up by solid-phase extraction (SPE). Residues were determined by reverse-phase high performance liquid chromatography (HPLC), coupled with tandem mass spectrometry (MS) (HPLC/MS/MS) (multiple reaction monitoring – MRM). Quantitation was done via external matrix matched external. Samples of 5.0 ± 0.1 gm of tissue from liver, kidney and muscle were separately put into 250 ml bottle containing 50 mL acetonitrile/acetone (80:20). After 30 minutes shaking, the samples were then centrifuged for 5 minutes at 2000 rpm. The supernatants were collected and filtered with 0.45µm syringe filter. Samples were kept at -20°C for one hour, centrifuged and supernatant were harvested in a 4-mL aliquots with 6-mL of 0.05 M potassium phosphate monobasic.

Evaluation of liver and kidney functions

Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein (TP) and albumin (ALB) were determined for liver function evaluation as described earlier (Kind and King, 1954; Reitman and Frankle, 1957; Buzanovskii, 2017). For renal function evaluation, Serum creatinine and uric acid were also determined using the method described by Henry (1974).

Statistical analysis

All data were analyzed by SPSS version 25 (Armonk, NY: IBM Corp). Results were expressed as a Mean ± SD. Independent samples t-test was used to test differences in each of the liver and kidney parameters between tested groups. Person correlation coefficient was used to test significant relationship between trace elements. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Antimicrobial (including antiprotozoal) drugs residues in the animal foods are the worldwide issue that need immediate attention of regulatory bodies to safeguard human health (Al-Ebrahim and El-Ghareeb, 2020). BPQ has been categorized as a lead hydroxynaphthoquinone compound that is broadly utilized as a specific antiprotozoa medication in different animals (Pasa, 2008; Müller et al., 2015). Thus an extraordinary attention must be driven to its harmful effect and its withdrawal time (Kachhawa et al., 2016). The recent study proposed that biogenic silicon nanoparticles are effective carriers to enhance the therapeutic efficacy of BPQ against resistant species of visceral leishmaniasis (Thapa et al., 2021). Results were acquired from intramuscular infusion of BPQ (2.5 mg/kg) given in single portion and samples were taken at 7th, 14th, 30th and 42th -day time points. There was no deaths were observed among animals during experiment. The results showed that the time of the experiment and type of organ together affected the residue level of BPQ (Table-1). Based on this, the data showed that the highest level of BPQ residues was found in liver at the first week of experiment (2614.03±4.92; $p<0.05$). At the 42 day time point post infusion, it was found that BPQ residues were only detected in liver tissues (26.37±2.59). A higher concentration of BPQ was observed in kidney and liver as compared to muscle ($p<0.05$) at 7th, 14th, and 30th day. In previous studies, these organs (kidney and liver) were also reported to have a higher antimicrobial residues as compared to muscle (Helal et al., 2020). Table-2 shows effect of BPQ on liver function at the various time points. It was found that there was no significant increment of serum ALP level at seventh and fourteenth days ($p>0.05$). However, at the 30th and 42nd day, it showed a high significance increment ($p<0.01$ and $p<0.05$, respectively) in the infused animals compared with the control animals. There was high noteworthiness in serum ALT at seventh ($p<0.01$), and at fourteenth day ($p<0.05$), however, there was no any observable significant increase at both days of 30th and 42th. The serum level of AST was a highly distinguished at only 30th and 42nd day post-infusion. Both serum total protein and albumin showed a significant increase at 14th, 30th, and 42nd days after BPQ infusion. Table-3 shows effect of BPQ on liver function at the various time points. The levels of both

Table 1: The concentration of buparvaquone (2.5mg/kg) in the tissues of rabbits harvested at 7th, 14th, 30th, and 42th using HPLC.

The time point	The organ	Mean ± SD
7th	Liver	2614.03± 4.92
	Kidney	2524.12 ± 7.94
	Muscle	942.96 ± 9.85
14th	Liver	1130.66 ± 17.79
	Kidney	1049.33 ± 17.21
	Muscle	528.18 ± 8.14
30th	Liver	101.99 ± 5.97
	Kidney	55.22 ± 2.44
	Muscle	14.19 ± 1.63
42th	Liver	26.37 ± 2.59
	Kidney	0.0000
	Muscle	0.0000

Table 2: Effect of BPQ (2.5mg/g) intramuscularly injected on liver functions at various time points.

Day	Group	ALT (u/l)	AST (u/l)	ALP (u/l)	TPT (gm/dl)	ALB(gm/dl)
7th	control	43.67 ± 3.21	24.33 ± 2.52	223.0 ± 2.65	5.23 ± 0.25	3.23 ± 0.32
	injected	68.00 ± 2.65	24.00 ± 6.93	222.33 ± 6.66	5.80 ± 0.87	3.83 ± 0.91
14th	control	30.67 ± 1.53	22.67±1.53	324.00 ± 3.61	6.20 ± 0.20	4.23 ± 0.25
	injected	36.33 ± 1.52	22.67 ± 1.15	295.33 ± 56.59	6.60 ± 0.10	3.67 ± 0.15
30th	control	48.00 ± 1.73	26.33 ± 3.05	372.0 ± 13.11	5.47 ± 0.31	3.73 ± 0.15
	injected	47.00 ± 4.36	33.33 ± 3.05	158.0 ± 36.72	7.3 ± 0.10	4.10 ± 0.10
42th	control	73.00 ± 2.00	35.33 ± 3.52	184.0 ± 3.61	6.23 ± 0.15	3.60 ± 0.44
	injected	70 .00	26.33 ± 2.52	174.0 ± 4.58	6.53 ± 0.15	3.27 ± 0.31

ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; TPT: Total protein test; ALB: Albumin.

Table 3: Effect of BPQ on kidney functions at different time points.

Day	Group	Creatinine(mg/dl)	Uric acid (mg/dl)
7th	control	0.80 ± 0.10	44.0 ± 3.61
	injected	1.37 ± 0.15	49.00 ± 1.0
14th	control	1.17 ± 0.15	44.66 ± 4.51
	injected	1.40 ± 0.10	35.00 ± 4.0
30th	control	1.20 ± 0.10	42.0 ± 2.65
	injected	1.37 ± 0.15	29.33 ± 4.04
42th	control	1.12 ± 0.08	35.0 ± 4.0
	injected	1.43 ± 0.15	39.66 ± 9.5

serum creatinine and uric acid were noticed higher at 7th and 42nd days than other time points after the drug injection. A recent published report showed that presence of the anti-theilerial drug BPQ in the milk and tissues of dairy cattle after treatment of this drug 2.5 mg/kg using two different commercial drugs (Butalex or Butaject). They found that the convergences of BPQ in milk declined with time post-treatment ($p<0.001$), however were over the limits of detection (LOD) in 11 of 25 bovines at Day 35 (Scott et al., 2016). The residues of BPQ as well as halquinol in food products derived from animal sources and available for human consumption could be reliably detected by liquid chromatography-tandem mass spectrometry (Zhang et al., 2017; Zheng et al., 2018).

Another investigation of BPQ deposits reported that drug deposits at 42th day were still existed in all tissues including liver, kidneys and fat except muscles (Bailey, 2013). The data of the current study was in the line with the previous mentioned studies that indicated that residues were pres-

ent for more than 42 days in liver.

CONCLUSION

It is concluded that, as suggested, liver of the infused animals with BPQ should not be used for human consumption for more than 42 days in rabbits; however, the other tissues can be safely consumed after 42 days from administration of BPQ.

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CONFLICT OF INTEREST

The authors report no declarations of interest.

AUTHORS CONTRIBUTION

All authors are equally contributed in Conceptualization, methodology, writing—original draft preparation, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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