Original Article

Pharm Sci Asia 2018; 45 (1), 45-53
DOI : 10.29090/psa.2018.01.045

Evaluation of clenbuterol-induced changes in blood biochemical parameters in white mice

N.T.B. Anh[1], N.T.M. Thuan[1]*
[1] Biochemistry Department of Pharmacy Faculty, University of Medicine and Pharmacy at Ho Chi Minh city, Viet Nam

ARTICLE INFO
Article history:
Received 30 September 2017
Received in revised form
14 November 2017
Accepted 24 November 2017

KEYWORDS:
Clenbuterol, Biochemistry parameters, White mice

ABSTRACT

Clenbuterol is a β_2- adrenergic agonist which is illegally used in animal production as a promoting agent. The accumulation of clenbuterol in edible tissues has potentially adversed effects that can induce to long-term metabolism disorders after clenbuterol withdrawal. Purpose of this study is to analysis effects of clenbuterol residues on some blood parameters in white mice. 68 mice were divided into 3 groups: control group (n = 27), clenbuterol group A (CA) (n = 20) and clenbuterol group B (CB) (n = 21). Clenbuterol groups were received clenbuterol 2 µg/kg orally once daily. After 8 weeks, mice in group A discontinued receiving clenbuterol and mice in group B continued receiving clenbuterol until 16 weeks. Liver, kidney and blood samples were collected at week 8 and week 16 for biochemistry parameter tests: plasma glucose, triglyceride, total cholesterol, total protein, creatinine concentrations; plasma AST and ALT activities. Results showed that oral clenbuterol administration at dosage 2 µg/kg once daily for 8 weeks significantly decreased ALT activities and elevated glucose concentrations. Clenbuterol-induced dyslipidemia might reverse after withdrawing using clenbuterol while hyperglycemia might potentially make a progress.

1. INTRODUCTION

Clenbuterol is a bronchodilator which is FDA-approved for treatment chronic obstructive pulmonary disease (COPD) in horse and non-lacting cattle[1,2]. Clenbuterol binds selectively to β_2-receptor in smooth muscles and consequently causing muscle relaxation. The recommended dose of clenbuterol for treatment COPD in veterinary is 0.8µg/kg twice daily[1,3]. At doses 5-10 folds higher than therapeutic dose, clenbuterol induces weight gain, increases muscle and decreases adipose tissue mass[4]. Due to its anabolic effects, clenbuterol is illegally used in animal production as a promoting agent and is abused by sportsmen for body-building[1,5,6].

The chemistry structure of clenbuterol is similar to derivations from endogenous catecholamines. The 3,5-dichloro groups prevent clenbuterol from being metabolized by catechol-O-methyltransferase (COMT), which results in its long half-life and its accumulation in animal tissues[4]. Researches on clenbuterol pharmacokinetics in men and animals reported that clenbuterol was well-absorbed when administered orally and excreted predominantly via urine[1,5]. After administering orally for 21 consecutive days at dosage of 10-20 times higher than the therapeutic dose, it was observed that clenbuterol accumulated in liver, bile, eye and other edible tissues[7]. Additionally, clenbuterol residues in animal tissues were found to be unaffected by boiling water and a range of cooking processes such as boiling, frying, roasting[5]. Consumption of clenbuterol-contaminated food can lead to

***Corresponding author:** minhthuan.nguyen49@yahoo.com

poison for human with many toxicity symptoms such as dizziness, tachycardia, tachypnea, tremors, vertigo, etc. These symptoms occurred between 0.5-3 hours after meal and maintained for 3-5 days in patients[8,9].

To control better the misuse of clenbuterol, the maximum residue limits (MRLs) of clenbuterol for liver and kidney in cattles and horses are accepted at 0.5µg/kg[3,9]. However, clenbuterol is used illcitly as a repartitioning agent and clenbuterol-contaminated food induced several acute intoxications in human[8,9]. In these cases, it was reported that clenbuterol residues in eddible tissues were higher 2,000 times than MRLs, and the amount of clenbuterol consumed was estimated about 230-300µg[9].

Previous studies evaluated the prolonged effects of clenbuterol on animals (mice, rats, pigs) at high concentrations (20-2,000 µg/kg body weight)[11-14], and the results showed that clenbuterol affected protein, glucose and lipid metabolism[15-18]. However, these studies were conducted for a short time. On the other hand, researches on effects of low-dose clenbuterol on animals were limited and only studied about repartitioning effects[19,20]. The aim of this study was to analyze the effects of low-dose clenbuterol on biochemistry parameters in white mice.

2. MATERIALS AND METHODS

2.1 Reagents and Samples

Clenbuterol hydrochloride was purchased from Sigma-Aldrich. 68 male *Swiss Albino* mice and animal food was purchased from Pasteur Institute (Ho Chi Minh city, Viet Nam). Reagent kits for determination of plasma AST, ALT activities and plasma glucose, total protein, total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol were the gifts from ELI-TechGroup. Reagent kits for plasma creatinine tests were purchased from AMS.

2.2 Animal care

68 male *Swiss Albino* mice (18-23 g) were bred in-house in a conventional colony, housed in controlled temperature (25^0C-27^0C) with relative humidity from 50-60% and a 12h-dark/light cycle. Water and animal food were given to the mice *ad libitum*. All the procedures were carried out under Animal Research Advisory Committee Guidelines. This study was performed with permission from scientific committee of university of medicine and pharmacy at Hochiminh city.

2.3 Study design

Mice were pre-fed for 2 days before the experiment to allow adaptation. 68 mice were randomly divided into 3 groups: control group (n = 27), clenbuterol group A (CA) (n = 20) and clenbuterol group B (CB) (n = 21). The average body weights between 3 groups were not significantly different at the beginning of experiment. All mice were weighted daily during the experimental period.

Clenbuterol hydrochloride was dissolved in distilled water to obtain clenbuterol concentration at 200 ng/ml and then was administered orally to 2 study groups CA and CB at dosage of 2 µg/kg body weight once daily. Control group were received equivalent volume of water instead of clenbuterol solution in the same manner.

After 8 weeks, CA group discontinued receiving clenbuterol solution while CB group continued receiving 200 ng/ml clenbuterol solution at dosage of 2 µg/kg body weight once daily until 16 weeks.

2.4 Sampling

Liver, kidney and whole blood samples were collected at the end of week 8 and week 16. Mice were anaesthetised by CO_2. Whole blood samples were obtained from the heart, then added into EDTA blood tubes and centrifuged at 4,500 x *g* for 10 min at room temperature. Plasma samples were used immediately for biochemistry parameter tests or stored at -5^0C until analysis (within 7 days).

2.5 Analysis method

Plasma creatinine concentrations were determined by the rate-blanked Jaffe assay (AMS). AST and ALT activities were determined by the IFCC method without pyridoxal phos-

phatase (ELITechGroup). Plasma total protein concentrations were determined by the Biuret method (ELITechGroup). Plasma triglyceride, glucose and total cholesterol concentrations were determined by the enzymatic-colorimetric method (ELITechGroup). HDL-cholesterol concentrations were determined by the precipitation method using phosphotungstic acid and Mg^{2+} (ELITechGroup). All these colorimetric methods were performed on Polker PPC 110 (Italia). LDL-cholesterol concentrations were calculated indirectly by using Friedewald formula. Livers and kidneys were isolated for macroscopy and then were weighted.

2.6 Statistical Analyses

All statistics were performed using R software (R Development Core Team, New Zealand, version 3.3.2). Results are presented as mean ± SEM. Student's t-test was used to evaluate the difference between groups. P-values < 0.05 were considered significant for all tests.

3. RESULTS

3.1 Effects of clenbuterol on body mass and the weights of viscera organs

It was observed that clenbuterol induced a rapid and significant increase in body mass after administering for 1 week ($p < 0.01$). Body weight gains between study groups and control group were significantly different within 8 weeks, but no significant difference was found in the next 8 weeks (from week 9 to week 16). From week 11 to week 16, the weekly body weight gains in the CB group unchanged, while the weekly body mass gains of CA group continued increased progressively (see Figure 1).

The kidney and liver weights at the end of week 8 and week 16 are shown in Table 1. Mean liver weight in study groups was greater than the control group at week 8 (CA+CB: 1.39 ± 0.06 g, control: 1.29 ± 0.04 g, $p = 0.17$), which slightly increased in the liver mass-to-body weight ratio (CA+CB: 4.42 ± 0.17%, control: 4.45 ± 0.07%, $p = 0.86$). However, these differences were not significant. At week 16, liver mass decreased in both CA and CB groups, but only the significant difference between CB group and control group was observed ($p = 0.004$). Mean kidney mass was significantly affected by clenbuterol after 8 weeks of experiment, but no significant difference in kidney weights among 3 groups was found at week 16 (see Table 1).

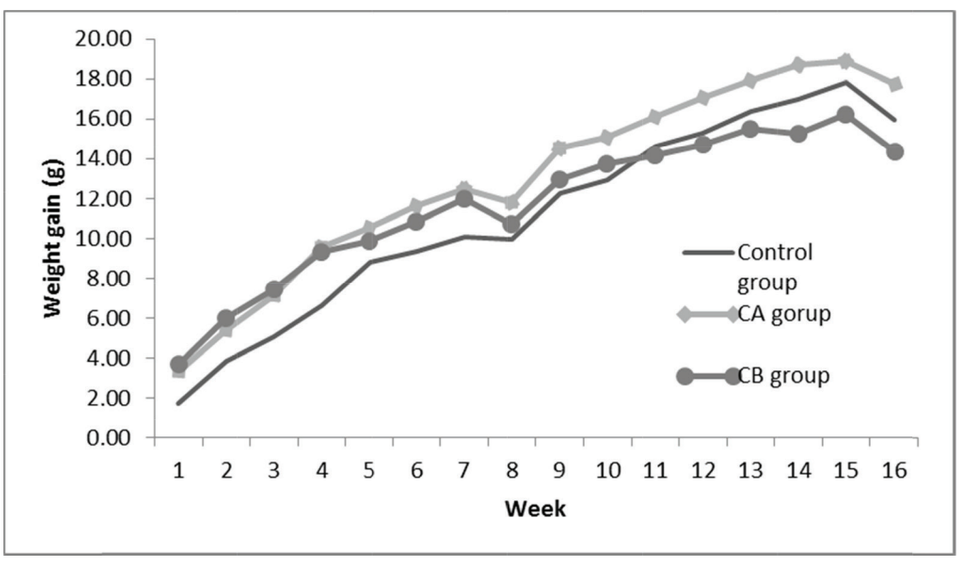

Figure 1. Weekly body weight gains of mice during experiment (16 weeks).

Table 1. Liver and kidney weights of mice at the end of week 8 and week 16

Organs	Week 8		Week 16		
	Control group	Group CA + CB	Control group	CA group	CB group
	(n = 13)	(n = 13)	(n = 14)	(n = 14)	(n = 14)
Liver	1.29 ± 0.04	1.39 ± 0.06	1.51 ± 0.05	1.43 ± 0.06	1.29 ± 0.05*
Kidney	0.27 ± 0.01	0.32 ± 0.02*	0.37 ± 0.02	0.38 ± 0.01	0.37 ± 0.02

*: p-value < 0.05

3.2 Effects of clenbuterol on biochemistry parameters

There was a significant increase in glucose concentration after using clenbuterol for 16 weeks between CB group and control group. Futhermore, the value of mean glucose concentration of CB group was over the normal threshold (106-278 mg/dl), suggested that clenbuterol caused hyperglycemia in mice. After a cessation of clenbuterol for 8 weeks, mean glucose concentration in CA group was found over the normal threshold and significantly higher than control group. However, there was no significant difference in mean total protein concentrations between study groups and control group at week 8 (CA+CB: 58.27 ± 4.06 mg/dl, control: 55.94 ± 2.15 mg/dl, p = 0.66), while significant increase in total protein concentration was found after using clenbuterol for 16 weeks in CB group versus control group (59.31 ±1.42 mg/dl, control: 51.50 ± 2.02 mg/dl, p <0.05). No abnormal value of plasma triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol concentrations were found in control mice at the end of week 8 and week 16. Compared to control group at week 16, plasma triglyceride and total cholesterol concentrations from group CB-mice could not be affected by clenbuterol, while mean plasma triglyceride concentration from group CA-mice was significantly higher. Mean HDL-cholesterol concentration decreased about 36% and mean LDL-cholesterol concentration increased about 34% after using clenbuterol for 8 weeks, but these changes were not significant comparing with control group. After withdrawing clenbuterol for 8 weeks (from week 9 to week 16), HDL-cholesterol and LDL-cholesterol values of mice in CA group were similar to that in control group. On the contrary, at week 16, in CB group, there was a significant decline in HDL-cholesterol value (CB: 53.55 ± 10.16 mg/dl, control: 90.97 ± 12.64 mg/dl, p = 0.033) and an increase of 18% in LDL-cholesterol value (CB: 73.95 ± 7.62 mg/dl, control: 62.51 ± 7.68 mg/dl), suggested that clenbuterol caused dyslipidemia in mice.

The results showed that clenbuterol had no effects on plasma AST activities (CA+CB: 2.77 ± 0.42 µkat/L, control: 2.76 ± 0.28 µkat/L, p =0.6471) but significantly decreased ALT activities after administering for 8 weeks (CA+CB: 1.20 ± 0.12 µkat/L, control: 1.69 ± 0.15 µkat/L, p =0.0171). Repeated oral administration of clenbuterol for a duration of 16 weeks induced significant decreases in values of both plasma AST activities (CB: 1.68 ± 0.12 µkat/L, control: 2.42 ± µkat/L, p =0.0028) and plasma ALT activities (CB: 1.08 ± 0.06 µkat/L, control: 1.69 ± 0.14 µkat/L, p =0.0016). Additionally, plasma ALT activities decreased after a cessation of clenbuterol for 8 weeks (CA: 1.06 ± 0.08 µkat/L, control: 1.69 ± 0.14 µkat/L, p =0.0012).

The values of plasma creatinine concentrations of clenbuterol-treated mice determined at the end of week 8 showed an increase by 9.5% versus the control mice (CA+CB: 0.81 ± 0.03 mg/dl, control: 0.74 ± 0.02 mg/dl, p = 0.0684). Noticeably, the values of control mice were in normal range (0.5-0.8 mg/dl) while that of clenbuterol-treated mice were beyond the normal threshold. However, this different was not significant. No significant difference was found among 3 groups at week 16 (Table 2). Mean plasma creatinine concentrations were

still higher than the normal threshold when administering clenbuterol repeatedly until 16 weeks, but would return to normal range after 8-week withdrawal of clenbuterol.

Table 2. Values of biochemistry parameters of mice at the end of week 8 and week 16

Biochemistry parameters	Week 8		Week 16			Normal range[21]
	Control group (n = 13)	Group CA + CB (n = 13)	Control group (n = 14)	CA group (n = 14)	CB group (n = 14)	
Glucose (mg/dL)	196.01 ± 24.46	333.23 ± 33.4*	215.01 ± 20.16	352.88 ± 31.06*	312.50 ± 25.42*	106-278
Total protein (mg/dL)	55.94 ± 2.15	58.27 ± 4.06	51.50 ± 2.02	55.43 ± 1.49	59.31 ± 1.42*	43-64
Total cholesterol (mg/dL)	154.48 ± 9.31	166.54 ± 10.56	166.43 ± 7.25	160.89 ± 10.04	146.80 ± 7.73	63-174
Triglyceride (mg/dL)	90.12 ± 10.61	100.20 ± 8.72	101.93 ± 5.42	119.83 ± 6.55*	105.11 ± 6.22	71-164
HDL-cholesterol (mg/dL)	112.17 ± 17.99	71.77 ± 13.84	90.97 ± 12.64	90.33 ± 16.14	53.55 ± 10.16*	
LDL-cholesterol (mg/dL)	64.81 ± 22.11	86.66 ± 10.61	62.51 ± 7.68	60.15 ± 15.65	73.95 ± 7.62	
AST activity (µkat/L)	2.76 ± 0.28	2.77 ± 0.42	2.42 ± 0.16	2.28 ± 0.29	1.68 ± 0.12*	1.15-3.18
ALT activity (µkat/L)	1.69 ± 0.15	1.20 ± 0.12*	1.69 ± 0.14	1.06 ± 0.08*	1.08 ± 0.06*	0.43-2
Creatinin (mg/dL)	0.74 ± 0.02	0.81 ± 0.03	0.73 ± 0.04	0.76 ± 0.05	0.81 ± 0.06	0.5-0.8

*: p-value < 0.05, clenbuterol-treated groups vs. control group.

4. DISCUSSION

The effects of β_2-agonists on glycemia were controversal. According to previous studies, after binding to β_2-receptor, β_2-agonists activated several sub-units of G protein family which consequently causes glucose-stimulated and hormone-stimulated insulin secretion[13,22]. When using at dosage of 10mg/kg and 100mg/kg consecutively in 30 days, clenbuterol induced a significant decline in glucose concentrations in mice[23]. According to Shujia J. Pan *et al.*, clenbuterol increased insulin action and the rate of glycogen synthesis in skeletal muscles and adipose tissues, which improved insulin resistance in concious obese Zucker rats[17]. However, in many case reports, glucose concentration increase was reported in patients who were hospitalized after clenbuterol administration or consumption of clenbuterol-contaminated food[1,2,24]. In our study, it was found that clenbuterol administration at dosage 2µg/kg for 8 weeks induced an augment of 70% in plasma glucose concentration in mice. In addition, there was a potential risk of clenbuterol-induced hyperglycemia in spite of the 8-week cessation. These results suggested that prolonged administration of clenbuterol would induce hyperglycemia in mice and this effect might be irreversible.

This could be explained since clenbuterol induced noradrenaline and glucocorticoid

(cortisol and corticosterone) release which induced hyperglycemia[25-27], clenbuterol could indirectly cause an increase in blood glucose concentration. A research of Wei Guo *et al.* showed that clenbuterol depleted glycogen deposition in hepatocytes and affected genes that are involved with glucose metabolism[16]. Therefore clenbuterol could induce a progress of hyperglycemia after 8-week withdrawal in mice.

The effects of clenbuterol and its mechanism on lipid metabolism were unclear. Hye-Kyeong Kim *et al.* reported that clenbuterol increased the rate of lipolysis and concomitantly decreased the rate of lipogenesis in adipose tissues[28]. In addition, it was found that clenbuterol down-regulated lipoprotein lipase, which hydrolyzes triglyceride in lipoproteins into glycerol and fatty acid[29]. In the present study, we observed that when orally administering clenbuterol to mice for 16 weeks at dosage 2 μg/kg, mean LDL-cholesterol concentrations were slightly increased and mean HDL-cholesterol concentrations were significantly decreased when comparing with control group, without changes in triglyceride and total cholesterol concentrations.

In mice, HDL-cholesterol amounted to approximately 70% and LDL-cholesterol amounted to about 25% of total total serum lipoprotein [30] therefore the HDL-cholesterol/LDL-cholesterol ratio in mice is > 1. In our study, the HDL-cholesterol/LDL-cholesterol ratios of control mice at the end of week 8 and week 16 were clearly > 1, while those of clenbuterol-treated mice were < 1, suggested that repeated administration of clenbuterol for a long time would lead to dyslipidemia. Additionally, the HDL-cholesterol/LDL-cholesterol ratio in clenbuterol-treated mice at the end of week 16 (0.72) was lower than that at the end of week 8 (0.83). However, after withdrawing clenbuterol for 8 weeks, HDL-cholesterol and LDL-cholesterol concentrations of mice in CA group were similar to control group and the HDL-cholesterol/LDL-cholesterol ratio was >1, suggested that dyslipidemia in mice was improved.

It has been well known that clenbuterol increases skeletal muscle and decreases adipose tissue mass when using at high dose[4]. The anabolic effect of clenbuterol was reported even when using at therapeutic dose[19,20]. In our study, clenbuterol was found to cause a significant increase in total plasma protein concentration after using for 16 weeks. This result was comparable to previous researches on mechanism of clenbuterol on protein metabolism, which clearly demonstrated that clenbuterol increased the rate of protein synthesis and decreased the rate of protein degeneration[15,31,32].

Researches on effects of clenbuterol on liver and hepatic function were limited. According to a study conducted by T. Gojmerac *et al.*, clenbuterol caused a significant increase in ALT activities and a slight decrease in AST activities in female pig after repeated administration for 28 days at dosage 10 μg/kg twice daily[11]. In patients who were hospitalized due to adversed effects of clenbuterol, increase in AST and ALT activities were reported[1,33]. Wei Guo *et al.* showed that clenbutrol decreased apoptosis in hepatocytes[16]. On the contrary, our study showed that oral administration of clenbuterol at dosage 2 μg/kg in mice decreased ALT activities and had no effect on liver weights after using for 8 weeks. We considered that the effects of clenbuterol on plasma ALT activities were irreversible as after the 8-week cessation. In addition, at week 16, plasma AST activities and liver weights significantly decreased in clenbuterol-treated mice when comparing with control mice. This result was comparable to a previous study, which showed a decrease in both AST activities and liver weight when using clenbuterol at high dose (100 mg/kg) in mice for 30 days[23].

According to Wilfred A. Nix *et al.*, increase in glycemia might lead to vitamin B_6 deficiency, which serves as a coenzyme of AST and ALT[34]. In both clenbuterol-treated groups, at the end of week 16, hyperglycemia was reported. Hence, we consider that AST and ALT activitiy decrease might be relative with clenbuterol-induced hyperglycemia. Further experiments need to be conducted to clarify the effects of clenbuterol on liver function as well as its mechanism.

For the effects of clenbuterol on renal function, results from previous researches were limited and unclear. Increase in plasma creatinine concentrations, even end-stage renal disease, was reported in patients who were hospitalized due to adversed effects of clenbuterol[35,36]. However, creatinine concentrations of some patients were in normal range[1,33]. Clenbuterol was found to accumulate in kidney and its residues were found on day 28 after clenbuterol withdrawal[4,7]. Consequently, it was considered that prolonged administration of clenbuterol had potential risk on renal function.

In our study, we found that plasma creatinine concentrations from group CB-mice were higher than normal threshold after clenbuterol withdrawal at week 16, while plasma creatinine concentrations from group CA- mice were in normal range. It was suggested that clenbuterol residues might reversibly affect renal function. However, since increase in plasma creatinine concentrations might be due to increase in protein degeneration, it is not sure whether clenbuterol induce renal dysfunction or not. Further analysis need to be carried out to confirm the effects of clenbuterol on kidney and to clarify its mechanism.

In conclusion, oral clenbuterol administration at 2µg/kg daily in mice (equivalent to 0.17µg/kg daily in human)[37] for a long time might affect kiney and liver function as well as glucose and lipid metabolism. Clenbuterol-induced dyslipidemia might reverse after withdrawing using clenbuterol while clenbuterol-induced hyperglycemia might potentially make a progress. Thus, prolonged consumption of clenbuterol-contaminated food or misuse clenbuterol for body-building have potential risks for dys-metabolism and organ dysfunctions. Since both hyperglycemia and dyslipidemia are risk factors of cardiovascular diseases, it is necessary to pay more attention to the use of clenbuterol in animal product and body-building areas.

5. ACKNOWLEDGMENTS

We wish to express our thanks to our colleges in Biochemistry Department, Pharmacy Faculty, University of Medicine and Pharmacy at Ho Chi Minh City for their supports to complete this study.

REFERENCES

1. Daubert GP, Mabasa VH, Leung VW, Aaron C. Acute clenbuterol overdose resulting in supraventricular tachycardia and atrial fibrillation. J Med Toxicol. 2007;3(2):56-60.
2. Huckins DS, Lemon MF. Myocardial ischemia associated with clenbuterol abuse:report of two cases. J Emerg Med. 2013;44(2):444-449.
3. The European Agency for the Evaluation of Medicinal Products. Veterinary Medicines and Information Technology Unit. Clenbuterol hydrochloride summary report. Committee for Veterinary Medicinal Products. 2000;1-6.
4. Zhao Z, Yao T, Qin Y, Yang X, Li J, Gu X. Clenbuterol Distribution and Residues in Goat Tissues After the Repeated Administration of a Growth-Promoting Dose. J Anal Toxicol. 2015;39(6):465-471.
5. Al-Majed AA, Khalil NY, Khbrani I, Abdel-Aziz HA. Clenbuterol Hydrochloride. Profiles Drug Subst Excip Relat Methodol. 2017;42:91-123.
6. Mazzanti G, Sotto AD, Daniele C, Battinelli L, Brambilla G, Fiori M, et al. A pharmacodynamic study on clenbuterol-induced toxicity: beta1- and beta2-adrenoceptors involvement in guinea-pig tachycardia in an in vitro model. Food Chem Toxicol. 2007;45(9):1694-1699.
7. Sauer MJ. Distribution and elimination of clenbuterol in tissues and fluids of calves following prolonged oral administration at a growth-promoting dose. J Vet Pharmacol Ther. 1995;18(2):81-86.
8. Barbosa J, Cruz C, Martins J, Silva JM, Neves C, Alves C, et al. Food poisoning by clenbuterol in Portugal. Food Additives and Contaminants. 2005;22(6):563-566.
9. Brambilla G, Cenci T, Flavia F, Galarini R, Marcrì A, Rondoni F, et al. Clinical and pharmacological profile in a clenbuterol

epidemic poisoning of contaminated beef meat in Italy. Toxicology Letters. 2000;114: 47-53.
10. Pleadin J, Vulic A, Persi N. b-adrenergic agonists: substances with anabolic effect in animals for meat production. Scientific and professional section. 2012;14:81-87.
11. Gojmerac T, Mandic B, Curic S, Pleadin J. Effects of repeated growth-promoting doses of clenbuterol on the hepatic function of female pigs. Vet Hum Toxicol. 2002;44 (5): 269-271.
12. Kaundal M, Sharma S. Diagnostic enzyme aided assessment of cytotoxicity induced by an acute dose of clenbuterol in mice tissues. Journal of Veterinary Science & Medical Diagnosis. 2014;3(1):1-7.
13. Kimple ME, Neuman JC, Linnemann AK, Casey PJ. Inhibitory G proteins and their receptors: emerging therapeutic targets for obesity and diabetes. Exp Mol Med. 2014; 46:1-9.
14. Shirato K, Tanihata J, Motohashi N, Tachiyashi K, Tomoda A, Imaizumi K. β2-Agonist Clenbuterol Induced Changes in the Distribution of White Blood Cells in Rats. J Pharmacol Sci. 2007;104(2):146-152.
15. Deutsch DAV, Abukhalaf IK, Wineski LE, Aboul-Enein HY, Pitts SA, Parks BA, et al. β-agonist-induced alteration in organ weights and protein content: comparison of racemic clenbuterol and its enantiomers. Chirality. 2000;12:637-648.
16. Liu Q, Zhang J, Guo W, Zhao Y, Hu X, Li N. Identifying lipid metabolism genes in pig liver after clenbuterol administration. Front Biosci (Elite Ed). 2012;4:2605-2616.
17. Shujia JP, Joe H, Zhenping D, Donovan F, Macheong L, John LI. Effects of clenbuterol on insulin resistance in conscious obese Zucker rats. Am J Physiol Endocrinol Metab. 2001;280:554-561.
18. Soppa GKR, Smolenski RT, Latif N, Yuen AHY, Malik A, Karbowska J, et al. Effects of chronic administration of clenbuterol on function and metabolism of adult rat cardiac muscle. Am J Physiol Heart Circ Physiol. 2005;288(3):1468-1476.
19. Kearns CF, McKeever KH, Malinowski K, Struck MB, Abe T. Chronic administration of therapeutics levels of clenbuterol acts as a repartitioning agent. J Appl Physiol. 2001;91:2064-2070.
20. Nolen-Walston RD, Moore CM, Barr CA, Tomlinson JE, Boston RC, Soma LR. Effect of long-term oral administration of a low dosage of clenbuterol on body fat percentage in working and nonworking adult horses. Am J Vet Res. 2015;76(5):460-466.
21. Danneman PJ, Suckow MA, Brayton C. The Laboratory Mouse. The Laboratory Animal Pocket Reference Series. 2nd ed. CRC Press; 2012
22. Wachter SB, Gilbert EM. Beta-adrenergic receptors, from their discovery and characterization through their manipulation to beneficial clinical application. Cardiology. 2012;122(2):104-112.
23. WHO food additives (Series: 57). Toxicological evaluation of certain veterinary drug residues in food. Prepared by sixty-six meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2006.
24. Waight M, McGuinness W. Case of low dose clenbuterol toxicity. BMJ Case Reports. 2016;2:151-57.
25. Illera JC, Silván G, Blass A, Martínez MM, Illera M. The effect of clenbuterol on adrenal function in rats. Analyst. 1998; 123(12):2521-2524.
26. Murugaiah KD, O'Donnell JM. Clenbuterol increases norepinephrine release from rat brain slices by a calcium- and receptor-independent mechanism. Res Commun Mol Pathol Pharmacol. 1994;86(3):311-324
27. Godinjak A, Iglica A, Burekovic A, Jusufovic S, Ajanovic A, Tancica I, et al. Hyperglycemia in Critically Ill Patients: Management and Prognosis. Med Arch. 2015 Jun; 69(3): 157–160.
28. Kim HK, Delle-Fera MA, Hausman DB, Baile CA. Effect of clenbuterol on apoptosis, adipogenesis and lipolysis in adipocytes. J Physiol Biochem. 2010;66(3):197-203.

29. Knych HK, Harrison LM, Steinmetz SJ, Chouicha N, Kass PH. Differential expression of skeletal muscle genes following administration of clenbuterol to exercised horses. BMC Genomics. 2016;17:596.
30. Tomikawa M, Nakayami T, Tawara K, Abiko Y. Effect of probucol on serum lipoprotein levels in normal and dyslipoproteinemic mice. Atherosclerosis. 1981;40: 101-113.
31. Ijiri D, Ishitani K, Shimamoto S, Ishimaru Y, Ohtsuka A. The effects of intraperitoneal clenbuterol injection on protein degradation and myostatin expression differ between the sartorius and pectoral muscles of neonatal chicks. Gen Comp Endocrinol. 2014; 206: 111-117.
32. Yang YT, McElligott MA. Multiple actions of b-adrenergic agonists on skeletal muscle and adipose tissue. Biochem J. 1989;261: 1-10.
33. Grimmer NM, Gimbar RP, Bursua A, Patel M. Rhabdomyolysis secondary to clenbuterol use and exercise. J Emerg Med. 2016;50(2): 71-74
34. Nix WA, Zirwes R, Bangert V, Kaiser RP, Schilling M, Hastalek U, et al. Vitamin B status in patients with type 2 diabetes mellitus with and without incipient nephropathy. Diabetes Res Clin Pract. 2015;107(1):157-165.
35. Hartung R, Gerth J, Funfstuck R, Grone HJ, Stein G. End-stage renal disease in a body builder: a multifactorial process or simply doping? Nephrol Dial Transplant. 2001;16: 163-165.
36. Quinley KE, Chen HY, Yang HS, Lynch KL, Olson KR. Clenbuterol causing non–ST-segment elevation myocardial infarction in a teenage female desiring to lose weight: case and brief literature review. Am J Emerg Med. 2016;34(8):1739.e5-7.
37. Shin JW, Seol IC, Son CG. Interpretation of animal dose and human equivalent dose for drug development. The Journal of Korean Oriental Medicine. 2010;31(3):1-7.