Original Article

Physiological Study of Dark Cocoa Intoxication Between Rabbits and Local Dogs

Salma Saeed abbas[1*], Nawras A. Alwan[2], Iqbal A. Al-Rufaei[3]

[1]University of Basrah, College of Education for Pure Sciences, Department of Biology.

[2]University of Basrah, College of Veterinary Medicine, Department of Physiology, Pharmacology and Biochemistry.

[3]University of Basrah, College of Science, Department of Biology.

Abstract

Intoxication of chocolate for small-animals may be threatening to life and associated with serious morbidity and mortality. This study was aims to compares the effect of dark cocoa ingestion in rabbits, local dogs and breeds dogs. The animals were randomly (4 rabbits or dogs/group) divided into two groups which include: Group- I (control group) was feed on normal diet, Group-II (G-treated) was feed on diet with 250 mg/Kg BW of dark cocoa (for 2 weeks to rabbits and 4 weeks for local dogs) while in breed dogs, While the serum and data of breed dogs taken from Veterinary Clinics. The results of clinical signs different in duration of occurrence intoxication between the animals used in this experiment, it more significantly effect in breed dogs and then male rabbits. Lier enzymes and oxidative enzymes elevation in rabbits after two weeks and four weeks in local dogs while in the breed dogs elevation within two days after ingestion of dark cocoa also significantly increased in lipid profile except HDL in all groups of treated in compared to the control groups also total protein, urea and creatinine elevation in all groups of ingestion cocoa with different duration of occurrence of intoxication. The current public presentation will focus on the latest findings on the effects of dark cocoa, its major constituents, and cocoa derivatives on selected biomarkers of toxicity and its duration in rabbits and dogs.

Keywords: dark cocoa, intoxication, rabbits, local dogs

Introduction

The main ingredient for chocolates and cocoa drinks are cacao beans, its rich in polyphenols that including catechins and procyanidins. The crude polyphenol fractions of cocoa have been reported to possess in vitro antioxidant activity and suppressive activity for LDL oxidation in cholesterol-fed rabbits. The Cocoa contains caffeine and methylxanthines. The methylxanthine amount that present it depends on the chocolate types: high cocoa content in chocolate, such as baked chocolate and dark cocoa, has a higher concentration of methylxanthines than milk chocolate 1,2. A toxic emergency for dogs is chocolate poisoning. The prevalence highly for this poisoning may be due to the fact that dogs often have access to a wide variety of foods containing chocolate with toxic ingredients including caffeine and methylxanthine-theobromine (~3:10ratio) 3,4. caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) are toxicities for dogs, that are contribute to the markers for clinical toxicity of chocolate, the first is the main reason because its concentration in chocolate is 3 to 10 times higher than that of caffeine and its half-life is much longer. Chocolate toxicity depends on the type of chocolate eaten but not on the amount of chocolate ingested. Different concentrations of methylxanthines founds in different products and range from low levels, as in white chocolate, to beans of cocoa, in the highest levels 5. Methylxanthines in different concentrations that are present in different products and range from lower concentration as in white cocoa to beans of cocoa is contains the high concentration 6,7.

Address for correspondence: Salma Saeed abbas, University of Basrah, College of Education for Pure Sciences, Department of Biology.

Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.03.105

How to cite this article: Saeed abbas S, Alwan N A, Al-Rufaei I A, Physiological Study Of Dark Cocoa Intoxication Between Rabbits And Local Dogs , J Pharm Negative Results 2022;13:698-702.

Aim of the study

This research was aim to study the comparative intoxication effect of dark cocoa on healthy status and some biochemical parameters between male rabbits and local\ male dogs.

Martials And Methods

Cocoa Powder

Cocoa powder was supplied from Saudi Arabia (Wardat Al Mashriq Food Factory) pure dark cocoa with 10-12%fat. Caffeine and theobromine have the lethal dose (LD50) in cocoa to dogs is about between 100–500 mg kg-1 8,9.

Animals and diets

Six male rabbits/group (range of Body Weight: 1.2-1.5kg) that are buy from Basrah Market at the age of 4-6 months. Six male dogs/group (body weight range: 2 -3 kg) were bring from Basrah street (age of 2 months). The animals that individually Living in a controlled environment (21– 25°C, 45–65% humidity, 12-hour dark and light cycle, more than 10 times ventilation/hour). Control animals received standard, commercially available diets. The animal's treatment groups received the admixture of the standard diets and cocoa powder by 10% (250 mg/ Kg BW). While the serum and data of breed dogs taken from Veterinary Clinics.

Study Design

The rabbits and groups of breed dogs and local dogs were divided into 2 groups as bellow (4 males / group):

1-The control group (GI): received the diets without cocoa.
2- The cocoa powder group (GII) received 250 gm/kg BW.

All groups were supplied with drinking tap H2O ad-libitum. Animals were clinically monitored and daily food intake measured. The measurement of body weights, biochemical parameters and oxidative stress were performs 2 weeks for rabbits (while for local dogs after 4 weeks) after the initiation of the study. The animals were euthanized by overdose of pentobarbital at the end of the administration study of cocoa powder. Ten ml of blood samples from anesthetized animals were collected by cardiac puncture using a 5 ml (sterile) syringe placed in test tubes without anticoagulant and then isolated serum by centrifugation (3000 rpm / 15 min), store them at -20°C until analysis, then sacrifice the animals to take the testes.

Measurements Biochemically

Biochemical measurements: Some biochemical measurements were made on serum after separation using special enzyme kits as follows:

Serum Aspartate-Aminotransferase (A S T) (U/l) & Serum Alanine-

Aminotransferase (A L T) Estimation (U / l)

The aminotransferase of aspartate and alanine is mensuration by monitoring the concentration of oxaloacetate hydrazone consisting of 2,4-dinitrophenylhydrazine 10.

Alkaline-Phosphatase (ALP) Estimation (U/l)

This measurement was performed using colorimetric determination of alkaline phosphatase activity 11.

Malondialdehyde -acid measurements (MDA)

The main end product of lipid oxidation is Malondialdehyde, and it will be performed in the blood serum according to the Yagi method 12. The basis of this principle is based on spectrophotometry. Thiobarbituric acid (TBA) reacts with MDA to form thiobarbituric acid reactive.

Estimation of Serum Super oxide dismutase activity (SOD)

The serum SOD which determined through SOD kit depends on Flohé & Günzler method 13.

Urea Measurement

Urea decomposes in the presence of water and urease to produce ammonia and nitrogen dioxide 11.

Measurement of serum creatinine

Creatinine is produced endogenously and released into body fluids at a constant rate and its levels in plasma and serum are maintained within narrow limits, and can be measured as indicator for filtration rate of glomerular (GFR) 14.

Measurement of plasma lipid levels

The plasma centrifuged from the EDTA- treated blood sample was examined for liver enzymes, oxidative enzymes, total cholesterol, triglyceride and lipoproteins. The total cholesterol, triglyceride and phospholipid were measured enzymatically using cholemetric kits 15.

Statistical Analysis

The results were expressed as Mean ± Standard Deviation (M±SD), the first and second experiments were analyzed by using independent T-test by SPSS version 22.0., the significant level was set on $p< 0.05$ 16.

Results

Clinical Signs: Symptoms of cocoa poisoning by ingestion may include restlessness, agitation, hyperactivity,

nervousness, shivering, vomiting, diarrhea, increased drinking and urination, elevation of heart rate, tremors of muscles, and seizures, and these signs were seen in rabbits after two weeks and these signs appear after 4 weeks in domestic dogs.

In Rabbits after 2 weeks

According to clinical signs appeared on male rabbits, these signs of cocoa intoxication the serum of euthanized animals were analyzed for estimation the liver enzymes and oxidative status (Table 1). The results significantly (P< 0.05) increased (ALT, AST and ALP) in treated group more than control group after 2 weeks of ingestion. Also MDA and SOD enzymes significantly (P< 0.05) increased in G treated rather than to group of control (Table 1).

Table 1: Effect of dark cocoa ingestion on liver enzymes and oxidative enzymes in rabbits

Groups	*ALT U/l*	*AST U/l*	*ALP U/l*	*MDA*	*SOD*
G-Control	95.25 ± 4.11	94.25± 2.21	83.25± 2.75*	3.01± 0.06	10.00± 0.81
G-Treated	152.50± 11.90*	138.00± 4.96*	128.00± 2.60*	5.09± 0.47*	13.85± 0.73*

* denote to the significant differences between groups (P<0.05).

The results represented in table (2) appeared significant increment in all parameters of lipid profiles (TC, TG, LDL and VLDL) except HDL was decreased significantly compared to control group.

Table 2: Effect of dark cocoa ingestion on lipid profile in rabbits

Groups	*TC mg/dl*	*TG Mg /dl*	*HDL Mg /dl*	*LDL Mg /dl*	*VLDL Mg /dl*
G-Control	38.30± 2.15	73.75± 2.75	95.94± 3.91	64.84± 1.44	17.06± 0.44
G-Treated	132.71± 7.35*	122.41± 1.29*	80.58± 3.83*	95.78± 2.14*	30.43± 0.93*

* denote to the significant-differences between each groups (P<0.05).

Table (3) showed the effect of dark cocoa ingestion on the kidney function (that include measurement total-protein (TP), urea and creatinine), total protein, urea and creatinine significantly (P<0.05) increased in treated group more than control group in male rabbits after 2 weeks of experiment.

Table 3: Effect of dark cocoa ingestion on Kidney function in rabbits

Groups	*TP g/ l*	*Urea. Mg /dl*	*Creatinine. Mbn kg/l*
G-Control	8.87± 0.61	42.39± 6.86	3.23± 0.60
G-Treated	9.87± 0.59*	61.95± 8.87*	5.59± 0.96*

* denote to the significant-differences between each groups (P<0.05).

In local dogs after 4 weeks

The results of measurement parameters after 4 weeks of ingestion of cocoa represented in table (4), the results of ALT, AST and ALP elevation significantly (P< 0.05) in treated group compared to control group. Also the results of SOD and MDA increased significantly in the cocoa group more than to control group.

Table 4: Effect of dark cocoa ingestion on liver enzymes and oxidative enzymes in male dogs

Groups	*ALT*	*AST*	*ALP*	*SOD*	*MDA*
G control	43.71 ±	19.14±	19.87±	5.38±	15.23±
	2.37	3.05	1.32*	0.32	0.56
G treated	96.67 ±	25.88±	23.21±	8.84±	18.51±
	1.95*	2.94*	0.82*	0.94*	0.89*

* denote to the significant differences between groups (P<0.05).

The data of results in table (5) appeared significantly (P< 0.05) elevation in lipid profile of treated group compared to control group: elevation TC,TG,LDL and VLDL but The level of HDL decrease significantly as compared to control group after 4 weeks of ingestion of dark cocoa in local dogs.

Table 5: Effect of dark cocoa ingestion on lipid profile in local dogs

Groups	*TC*	*TG*	*HDL*	*LDL*	*VLDL*
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
G-Control	96.07±	92.43±	89.04±	99.08±	21.13±
	13.90	7.54	2.29	1.98	1.36
G-Treated	142.08±	144.57±	72.12±	183.43±	29.79±
	44.93*	2.73*	5.97*	19.41*	1.07*

* denote to the significant differences between groups (P<0.05).

Also the results of total protein, urea and creatinine elevation significantly after 4 weeks of treated rather than to control group in local dogs and differs from rabbits that appeared the intoxication signs after 2 weeks of experiment and also that in breed dogs that appear these signs after 2 days of ingestion.

Table 6: Effect of dark cocoa ingestion on Kidney function in local dogs

Groups	*Total protein* *g / l*	*Urea* *Mg / dl*	*Creatinine* *Mbn kg/ l*
G-Control	9.65±	40.88 ±	4.11 ±
	0.74	7.41	0.54
G-Treated	11.43±	77.08 ±	7.07±
	0.87 *	9.32*	1.22*

* denote to the significant differences between groups (P<0.05).

Discussion

Chocolates are derived of roasted the plant seeds, theobroma cacao, and the alkaloids methylxanthine theobromine are the main toxic components and also caffeine. In humans, methylxanthine can easily digest and excrete, and the half-life of theobromine is two to three hours. However, in dogs, it is absorbed slowly, with metabolism in the liver and recirculation outside the liver before being excreted in the urine. The half-life of theobromine in dogs is about eighteen hours 7.

Theobromine has the lethal dose reported between 100-500 mg / kg BW in dogs. However, not all chocolate contains the same amount of theobromine: cocoa powder and regular chocolate have the highest concentrations (20 mg/g and 15 mg/g), while milk chocolate has a much lower percentage (2 mg/g), and chocolate albicans has the lowest concentration

(0.1 mg/g). Thus, less than 100 g of plain chocolate may be fatal for a 10 kg dog 18. Theobromine mainly affects the central nervous system, cardiovascular system, respiratory system, and also has a diuretic effect. Polydipsia and bloody vomiting are the first signs of dog poisoning. Another signs may include hyperexcitability, hyperexcitability, tachycardia, and excessive panting, ataxia, and muscle fibrillation. Effects may progress to irregular heartbeat, seizures, and death. Most symptoms will begin to appear within two hours of ingestion, but because theobromine is slowly metabolized, it can take up to 24-48 hours and up to three days to recover. Therefore there is no specific antidote; supportive management includes vomiting induction and administration of oxygen, activated charcoal, and I/V fluids 19.

Although it is relatively safe to give your pet a small treat of chocolate once in a while, all dogs are at risk for chocolate, and a safer alternative is to give your pet a special "pet chocolate" that does not contain theobromine (which causes obesity). As a final thought, careful gardeners who want to spread mulch in their garden in spring and summer should be aware that cocoa shell mulch also contains very high levels of theobromine (25 mg/g). Because it has a distinct chocolate scent, it may be attractive to dogs but can be fatal 20. Toxic principles are theobromine and caffeine, in chocolate poisoning, which are easily absorbed from the gastrointestinal tract and widely distributed throughout the various organs of the body. In the liver, it is metabolized and undergoes enterohepatic recycling, so the elevation of AST, ALT and ALP enzymes was also shown to increase oxidative enzymes similar to that result in this study. The metabolites of methylxanthine are excreted in the urine. The half-lives of theobromine and caffeine in dogs are 17.5 and 4.5 hours, respectively. These compounds competitively inhibit cellular adenosine receptors, resulting in tachycardia, CNS stimulation and diuresis, and they also increase intracellular calcium levels by increasing cytosolic calcium entry and inhibiting intracellular calcium sequestration by the sarcoplasmic reticulum of the striated muscle. The net effect is to increase strength and contractility of skeletal and cardiac muscles. Methylxanthines may also compete for benzodiazepine receptors within the central nervous system and inhibit phosphodiesterase, which leads to increased levels of cyclic adenosine monophosphate (cyclic AMP), and may also increase circulating levels of adrenaline and norepinephrine 21. Theobromine and its derivatives act as smooth muscle relaxants, diuretics, cardio stimulants, and coronary vasodilators. The diuretic effect of theobromine, which results from an increase in the glomerular filtration rate and the reabsorption of sodium and water, is more sustained than that of theophylline, but is less pronounced. Theobromine as an undesirable substance in animal feed 1 Scientific opinion of the Committee on Pollutants in the Food Chain 22. There are no previous studies on the effect of dark cocoa on rabbits, the period of intoxication, and the duration of the dose at which intoxication occurs. We also noticed that rabbits are also poisoned by this substance, which are faster than domestic dogs and symptoms appear faster than those in them.

Compliance with Ethical Standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval for this research was obtained from the University of Basrah, College of Education for Pure Sciences, Department of Biology Local Committee

References

Abbe Maleyki, M. J. and Ismail, A. (2008). Polyphenols in Cocoa and Cocoa Products: Is There a Link between Antioxidant Properties and Health?. Molecules; 13(9): 2190–2219.

Rios L.Y., Gonthier M.P., Remesy C., Mila I., Lapierre C., Lazarus S.A., Williamson G., Scalbert A. (2003). Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. Am. J. Clin. Nutr.;77:912–918.

Kovalkovičová, N. Šutiaková, I.; Pistl, J. and Šutiak, V. (2009). Some food toxic for pets. Interdiscip Toxicol.; 2(3): 169–176.

Agudelo, C.F.; Filipejova, Z. and Schanilec, P. (2013). Chocolate ingestion-induced non-cardiogenic pulmonary oedema in a puppy: a case report. Veterinarni Medicina, 58(2): 109–112.

Luiz JA, Heseltine J (2008): Five common toxins ingested by dogs and cats. Compendium on Continuing Education for the Practicing Veterinarian; 30: 578–587.

Gwaltney-Brant, S. (2001): Chocolate intoxication. Veteri nary Medicine 96:108–111.

Jansson, D.S.; Galgan, V.; Schubert, B.; Hard, af. And Segerstad, C. (2001): Theobromine intoxication in a red fox and a Eu ropean badger in Sweden. Journal of Wildlife Diseases; 37: 362–365.

Albretsen, J.C. (2004): Methylxanthines. In: Plumlee KH.(ed.): Veterinary Clinical Toxicology. 1st ed. Mosby Inc., St. Louis, MO. 322–326.

Carson, T.L. (2006): Methylxanthines. In: Peterson ME, Tal cott PA (eds.): Small Animal Toxicology. 2nd ed. Saunders, St. Louis, MO. 845–852.

Schumann, G. and Klauke, R. (2003). New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: Preliminary upper reference limits obtained in hospitalized subjects. Clin. Chim. Acta; 327(1-2): 69-79.Tietz, N.W. (2006). Clinical guide to laboratory test. 4th ed. Publ. U.S.; 638- 9 ET: 1062-1065.

Yagi, K. (1998). Serum malondialdehyde measurements. Free Rad. Antiox.

Prot.; 108:101-106.

Flohé, L. and Günzler, W.A. (1984). Assays of glutathione peroxidase.

Metho. Enzymol.;105:114-121.

Peake, M. J. and Whiting M. (2006). Measurement of Serum Creatinine – Current Status and Future Goals. Clin. Biochem. Rev.; 27 :173-175.

Crook, D. (1996). A survey of biases in the measurement of plasma lipid and lipoprotein concentrations in 32 lipid clinics in the UK. Ann. Clin. Biochem.; 33: 82-83.

Abo-Allam, R.M. (2003). Data statistical analysis using SPSS Program. 1st ed. Publ. for the U. Cairo Pp.: 32-54.

Finlay, F. and Guiton, S. (2005). Chocolate poisoning. BMJ. ; 331(7517): 633.

Reddy B. S.; LSS,V.R. and Sivajothi, S. (2013). Chocolate poisoning in a dog. Intern. J.of Veteri. Health Sci. & Res., 01(03),16-17.

Fiona Finlay,2005. Chocolate poisoning. B.M.J. ; 331(7517): 633.

Gwaltney-Brant , S.M.(2021). Chocolate toxicosis in animals. MSD Vet.

Manual. Pp.: 1-3.

Weingart, C.; Hartmann, A. and Kohn, B. (2021). Chocolate ingestion in 156 dogs. Journal of Small Animal Practice, Pp.:1–5

Jan Alexander, Diane Benford, Andrew Cockburn, Jean-Pierre Cravedi, Eugenia Dogliotti,Alessandro Di Domenico, Maria Luisa Férnandez-Cruz, Peter Fürst, Johanna Fink-Gremmels,Corrado Lodovico Galli, Philippe Grandjean, Jadwiga Gzyl, Gerhard Heinemeyer, NiklasJohansson, Antonio Mutti, Josef Schlatter, Rolaf van Leeuwen, Carlos Van Peteghem and Philippe Verge (2008). Theobromine as undesirable substances in animal feed1 Scientific Opinion of the Panel on Contaminants in the Food Chain. The EFSA Journal. 725: 1-66.