Original Article

Physiological Study of Dark Cocoa Intoxication Between Rabbits and Local Dogs

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Abstract

Intoxication of chocolate for Small animals may be dangerous to life and be linked to serious illness and death. 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 dietwith 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 animalsused 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 all groups had a rise in total protein, urea, and creatinine 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 toxicityand its duration in rabbits and dogs.

Keywords: dark cocoa, intoxication, rabbits, local dogs

Introduction

The main ingredient Cacao beans, which are used to make chocolate and cocoa drinks, are high in polyphenols, such as 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: Chocolate with a lot of cocoa, like baked chocolate or dark cocoa, has more methylxanthines than milk chocolate. 1,2. Chocolate poisoning is a very dangerous emergency for dogs. Dogs often have access to a wide range of foods that contain chocolate with toxic ingredients like caffeine and methylxanthine. This may be one reason why this poisoning happens so often. -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 It is 3 to 10 times more concentrated in chocolate than caffeine, and its half-life is much longer. How dangerous chocolate is depends on the kind of chocolate eaten, not how much chocolate is eaten. There are different amounts of methylxanthines in different products. White chocolate has the least amount, while cocoa beans have the most. 5. There are different amounts of methylxanthines in different products. For example, white cocoa has a lower concentration than cocoa beans, which have a high concentration. 6,7.

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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 cycle of dark and light, more than 10 times per hour of air flow). Standard, commercially available food was given to the animals in the control group. The animals' diets were mixed with cocoa powder by 10% (250 mg/Kg BW) and given to the treatment groups. While the serum and information about breeds of dogs were 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 of the groups had free access to water from the tap. Clinical tests were done on the animals, and their daily food intake was 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: Using enzyme kits, some biochemical measurements were made on the serum after it was separated :

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

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

The concentration of oxaloacetate hydrazone made of 2,4-dinitrophenylhydrazine 10 is used to measure the aminotransferase of aspartate and alanine.

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

This measurement was performed using colorimetry to measure the activity of alkaline phosphatase 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

When urea is mixed with water and urease, it breaks down into ammonia and nitrogen dioxide.

Measurement of serum creatinine

Creatinine is made by the body and released into body fluids at a steady rate. Its levels in plasma and serum stay within a narrow range and can be used to measure the filtration rate of the glomeruli (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 increased more in the treated group than in the control group (P0.05) 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*

* show that there are big differences between the 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*

* point out the big differences between the 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

Theobroma cacao plant seeds are roasted to make chocolate. Methylxanthine and theobromine, two alkaloids, and caffeine are the most dangerous parts of chocolate. Theobromine has a half-life of two to three hours and is easy for humans to break down and get rid of. But dogs take longer to absorb it., with metabolism in the liver andrecirculation outside the liver before being excreted in the

urine. The half-life of theobromine in dogs is about eighteenhours 7.

The amount of theobromine that can kill a dog is between 100 and 500 mg/kg BW. But not all chocolate has the same amount of theobromine: cocoa powder and regular chocolate have the most (20 mg/g and 15 mg/g), while milk chocolate and chocolate albicans have the least (2 mg/g and 1 mg/g, respectively). concentration

(0.1 mg/g). So, a 10 kg dog could die from less than 100 g of plain chocolate. Theobromine mostly affects the central nervous system, the heart, and the lungs, and it also makes you pee more. If a dog has been poisoned, it will start to drink a lot and throw up blood. There may also be hyperexcitability, tachycardia, and a lot of panting, as well as ataxia and muscle fibrillation. The effects can get worse and lead to a shaky heartbeat, seizures, and even death. Most symptoms will start to show up within two hours, but because theobromine is hard to break down, it can take anywhere from 24 to 48 hours to three days to feel better. 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

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