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Cocoa intoxication in domestic and street dogs: A comparative study

Jala A S Alahmed[1], Iqbal A. Al-Rufaei[2], and Nawras A. Alwan[1]

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[1] Department of Physiology Pharmacology and Chemistry, College of Veterinary Medicine, University of Basrah, Basrah, Iraq. ORCID: 0000-0003-1033-2626, 0000-0001-8101-2545.
[2] Department of Biology, College of Science, University of Basrah, Basrah, Iraq, ORCID: 0000-0003-3345-6859

Corresponding author: jala.salman@uobasrah.edu.iq

Abstract: Chocolate intoxication can be life-threatening to dogs and cats with high morbidity and mortality. The current study aims to compare the toxic effect of dark chocolate on domestic dogs versus street dogs. Eighteen male dogs were used in this study, nine of each of domestic dogs and street dogs. The experiment continued for seven consecutive days. After three days, blood samples were collected as a control, and one dog from each group was sacrificed for negative control histopathological purposes. The same animals of both groups received a mixture of the standard diets and cocoa powder (1g / Kg BW / day) for four days. Clinical signs appeared faster in domestic dogs than in street dogs. Liver enzymes and oxidative stress indicators were elevated after four days in both street dogs the domestic dogs but not in their control groups. Furthermore, there was a significant increase in lipid profile except for HDL in the groups treated with cocoa compared to the control groups. The same results were noticed regarding total protein, urea, and creatinine since they significantly increased against their controls. In conclusion, cocoa is toxic to both domestic and street dogs in the same way except for the starting of the clinical signs where the domestic dogs were affected earlier than street dogs.

Keywords: cocoa intoxication, street dogs, domestic dogs.

1. Introduction

Chocolate (cocoa) is one of the most common toxic foods ingested by pet dogs (Luiz & Heseltine, 2008). It contains some toxic substances for many types of animals including canine and feline (Gwaltney-Brant, 2001). The main toxic components of chocolate are methylxanthines i.e., theobromine, theophylline, and caffeine (Haydock, 2012).

Theobromine shows moderate acute toxicity in dogs which are considered to be more susceptible to methylxanthines than rodents (Jalil & Ismail, 2008). Contrary to the other methylxanthines, theobromine has no clinical significance with a mild effect on the central nervous system. It has a weak antagonistic activity at adenosine receptors (Haydock, 2012). Animals show different signs of toxicity depending on many factors including age, gender, animal type, and chocolate type. For instance, dark chocolate has greater theobromine content than milk chocolate. Dogs exposed to 20 mg/kg of theobromine can produce mild clinical signs of toxicity, while a dose of 40 to 50 mg/kg can cause severe signs, and 60 mg/kg can cause seizures (Gwaltney-Brant, 2001). The LD50 of theobromine in dogs is 100-200 mg/Kg which means that a small bar of dark chocolate (100-200 grams) is considered to be hazardous and fatal for dogs (Hudd, 1997). Theobromine stimulates the CNS and the cardiovascular system resulting in different signs including seizures, tachycardia, and arrhythmias (Osweiler & Wilkins, 1996). Moreover, it causes testicular-targeting reproductive toxicity in rodents and dogs (Jalil & Ismail, 2008).

Symptomatic and supportive management have priority in the treatment of chocolate poisoning because there is no specific antidote. The treatment includes induction of vomiting, administration of activated charcoal, oxygen therapy, and fluids therapy (Luiz & Heseltine, 2008). Preventing exposure is key to reducing the occurrence of poisoning. Therefore, it is important to raise pet owners' knowledge regarding potentially hazardous food (Gwaltney-Brant, 2001; Luiz & Heseltine, 2008).

2. Materials and Methods

The cocoa powder was purchased from local markets which is produced by Wardat Al Mashreq Food Factory (Riyadh, KSA).

2.1. Experimental design:

Eighteen male dogs were used in the current study, half of them were street dogs and the other half were domestic. The average body weight was 8-10 kg with an approximate age of 10-12 months. The street dogs were chased from Basrah city streets while the domestic dogs were purchased from veterinary shops and clinics.

The animals were individually housed in a controlled environment at the animal house of the College of Veterinary Medicine University of Basrah, Basrah, Iraq. Each group of animals received commercially available standard diets with drinking water ad libitum for seven days. After three days blood samples were collected as a control and one dog from each group was sacrificed for negative control histopathological purposes. Then, the same animals of both groups received a mixture of the standard diets and cocoa powder (1g/Kg/BW/day) for four days. The total daily amount of cocoa was approximately 10g/dog (32 mg of theobromine).

2.2. Biochemical Measurements:

Some serologic measurements were performed by specific kits using a spectrophotometer (Shimadzu, Tokyo, Japan) as follows:

2.2.1.Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT):

Aspartate and alanine aminotransferase were measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine (Salma Saeed *et al.,* 2022). Values were expressed as Units/liter (U/l).

2.2.2.Serum Alkaline Phosphatase (ALP) (U/l):

This estimation was done by using the colorimetric determination of alkaline phosphatase activity (Salma Saeed *et al.,* 2022).

2.2.3.Serum Malondialdehyde measurement (MDA):

Malondialdehyde is the main end product of lipid peroxidation. MDA levels were estimated in serum when thiobarbituric acid (TBA) reacts with MDA to form reactive substances that measured were using a spectrophotometer (Aguilar & Borges, 2020).

2.2.4.Serum Super oxide dismutase (SOD):

The serum SOD was determined by the SOD assay kit according to Flohé & Günzler method using a spectrophotometer (Flohé & Günzler, 1984).

2.2.5.Urea Measurement:

Urea hydrolyzed in the presence of water and urease to produce ammonia and nitrogen dioxide (Baum *et al.,* 1975). A spectrophotometer was used to measure blood urea levels.

2.2.6.Serum Creatinine Measurement:

Creatinine is endogenously produced and released to body fluids at a stable rate and its plasma and serum levels are maintained within narrow limits, it can be measured as an indicator of glomerular filtration rate (GFR) (Baum *et al.,* 1975).

2.2.7.Total serum protein level:

Serum proteins (Albumin and globulin) were measured in total as an indicator of kidney and liver functions using a spectrophotometer.

2.2.8.Measurement of plasma lipid levels:

The total cholesterol, triglyceride, and phospholipid were measured enzymatically using colorimetric kits and measured by spectrophotometer.

Histopathology:

Samples of the liver and kidney were collected and the processes of sectioning and staining were performed by the Department of Anatomy and Histology at the College of Veterinary Medicine, University of Basrah.

Statistical Analysis:

The results were expressed as mean ± standard deviation (Mean ± SD). Data were analyzed by student t-test and one-way ANOVA using the GraphPad Prism 8 software. P values are presented as asterisks when (* P< 0.05, ** P<0.01, *** P<0.001, and **** P<0.0001).

3. Results

3.1. Clinical Signs:

The symptoms of toxicity appeared in domestic dogs within 8-24 hours of exposure to cocoa, while it was delayed to reach up to 72 hours in some street dogs. These signs include restlessness, agitation, hyperactivity, nervousness, shivering, vomiting, diarrhea, increased water consumption, frequent urination, tachycardia, muscle tremors, and seizures. The clinical signs that appeared on street dogs were less severe than those of domestic dogs.

3.2. The liver function tests:

Serum levels of ALT, AST, and ALP were measured in both groups of domestic and street dogs with and without cocoa intake (n=8). For domestic dogs, the mean values ± SD (IU/L) of cocoa treated group of all three parameters (ALT 113.5 ± 8.401, AST 115.0 ± 6.633, and ALP 112.5 ± 5.155) were significantly higher than negative controls (ALT 93.75 ± 7.851, AST 94.25 ± 3.615 and ALP 79.38 ± 3.335) as can be seen in Fig. 1 A, B and C respectively. Similar findings were noticed for street dogs where the mean values ± SD of cocoa treated group (ALT 152.8 ± 11.23, AST 138.0 ± 3.780, and ALP 128.0 ± 2.0) were significantly higher than control values (ALT 95.00 ± 3.464, AST 94.38 ± 1.685 and ALP 83.50 ± 2.138) respectively (Figure 1).
There were no differences between the values of control groups of domestic and street dogs for the above three parameters. Given these findings, it is concluded that domestic and street dogs' liver enzymes responded in the same manner to the cocoa exposure.
Repeated measure (RM) one-way ANOVA was used to compare the groups, corrected with Sidak's multiple comparisons tests.

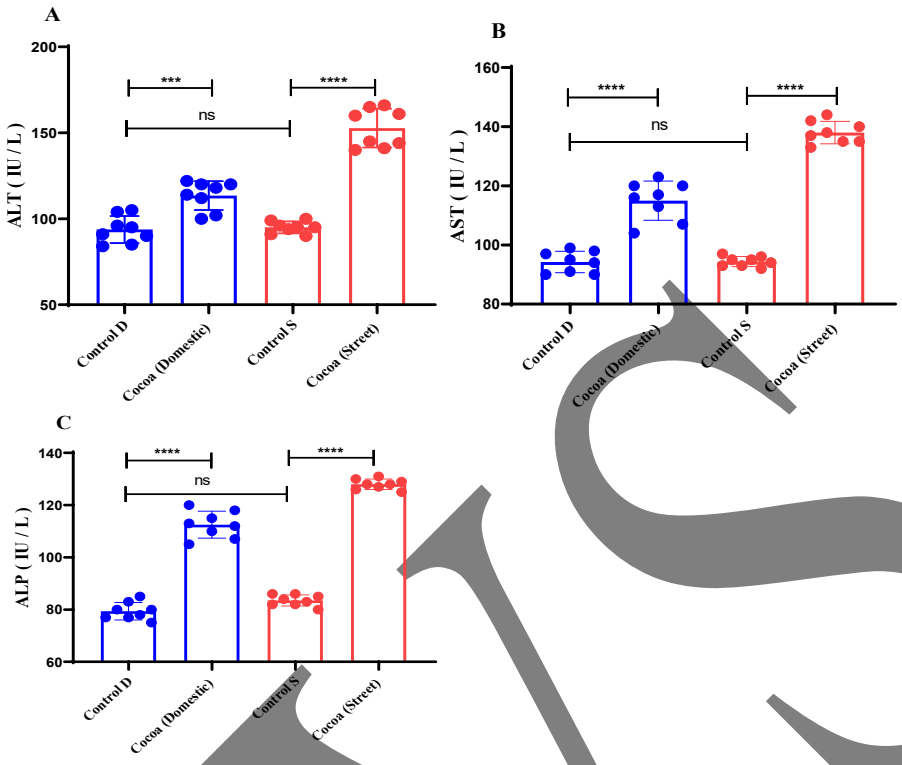

Figure 1: Effect of cocoa intake on liver enzymes. Values are expressed as means ± SD, (ns) means non-significant, n=8.

3.3. Oxidative stress tests:

Superoxide dismutase enzyme (SOD) levels and Malondialdehyde (MDA) levels were used as biomarkers of oxidative status in this study. Both tests were expressed as means ± SD and were measured in (IU /L). Regarding SOD outcomes, the mean values of cocoa treated in domestic dogs were significantly more than the control ($P<0.05$) (Figure 2A). The same figure shows that cocoa treated group of street dogs had higher values than the control ($P<0.05$). ANOVA comparison between the control values of domestic (control D) and street dogs (control S) revealed that there are no significant differences. On the other hand, there is a significant difference between the MDA control D mean values and control S values which was higher ($P<0.001$) (Figure 2B). In addition, there is a significant increase ($P<0.01$) in the MDA values of cocoa-treated domestic dogs compared to control D. The same result can be noticed in the MDA values of cocoa-treated street dogs and control S ($P<0.01$), as in Figure 2B. The above results confirm that cocoa intake by both types of dogs led to the production of ROS and subsequently increase the values of both SOD and MDA. RM one-way ANOVA was used to compare the groups, and it was corrected with Sidak's multiple comparisons tests.

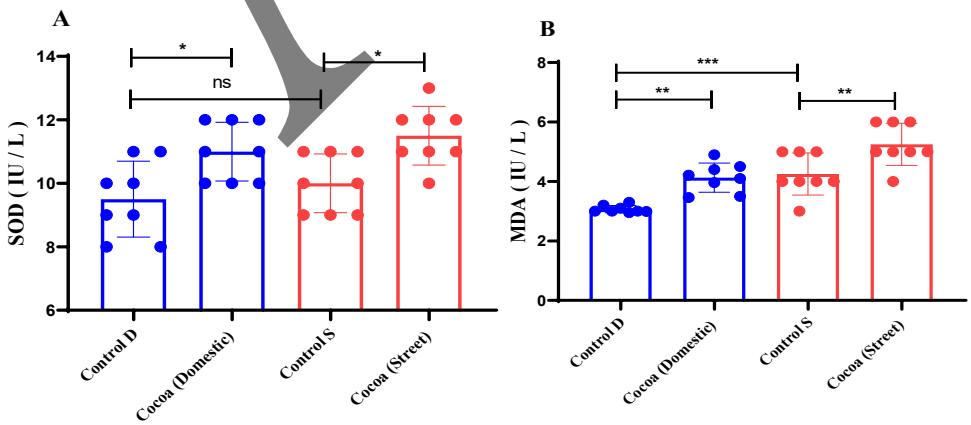

Figure 2: Effect of cocoa on oxidative stress parameters. Values are means ± SD, (ns) is non-significant, n=8.

3.4. Lipid profile tests:

In this study lipid profile was investigated as a potential target of cocoa toxicity, including total cholesterol (TC), total glycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Figure 3 A shows that the total cholesterol level (mg/dL) of cocoa treated group in both domestic and street dogs is significantly higher than their control groups ($P<0.0001$). Although TC mean values and SD of cocoa-treated groups of domestic dogs (78.88 ± 6.3) and street dogs (72.39 ± 6.2) are comparable, their controls are significantly different ($P<0.0001$) where the street dogs' TC values are lower than the domestic dogs. Similar observations are noticed in all other parameters except HDL since cocoa treatment significantly increased the values of TG, LDL, and VLDL as in Figure 3B, D, and E, respectively. Looking at Figure 3C, it is apparent that the HDL of cocoa-treated groups of both domestic and street dogs is significantly less than their controls ($P<0.0001$). There is no significant difference between the HDL control of domestic and street dogs. In general, cocoa exposure in both groups of dogs led to an increase in the lipid profile values in comparison with their controls except for HDL which was significantly decreased. RM one-way ANOVA was used to compare the groups, corrected with Sidak's multiple comparisons tests.

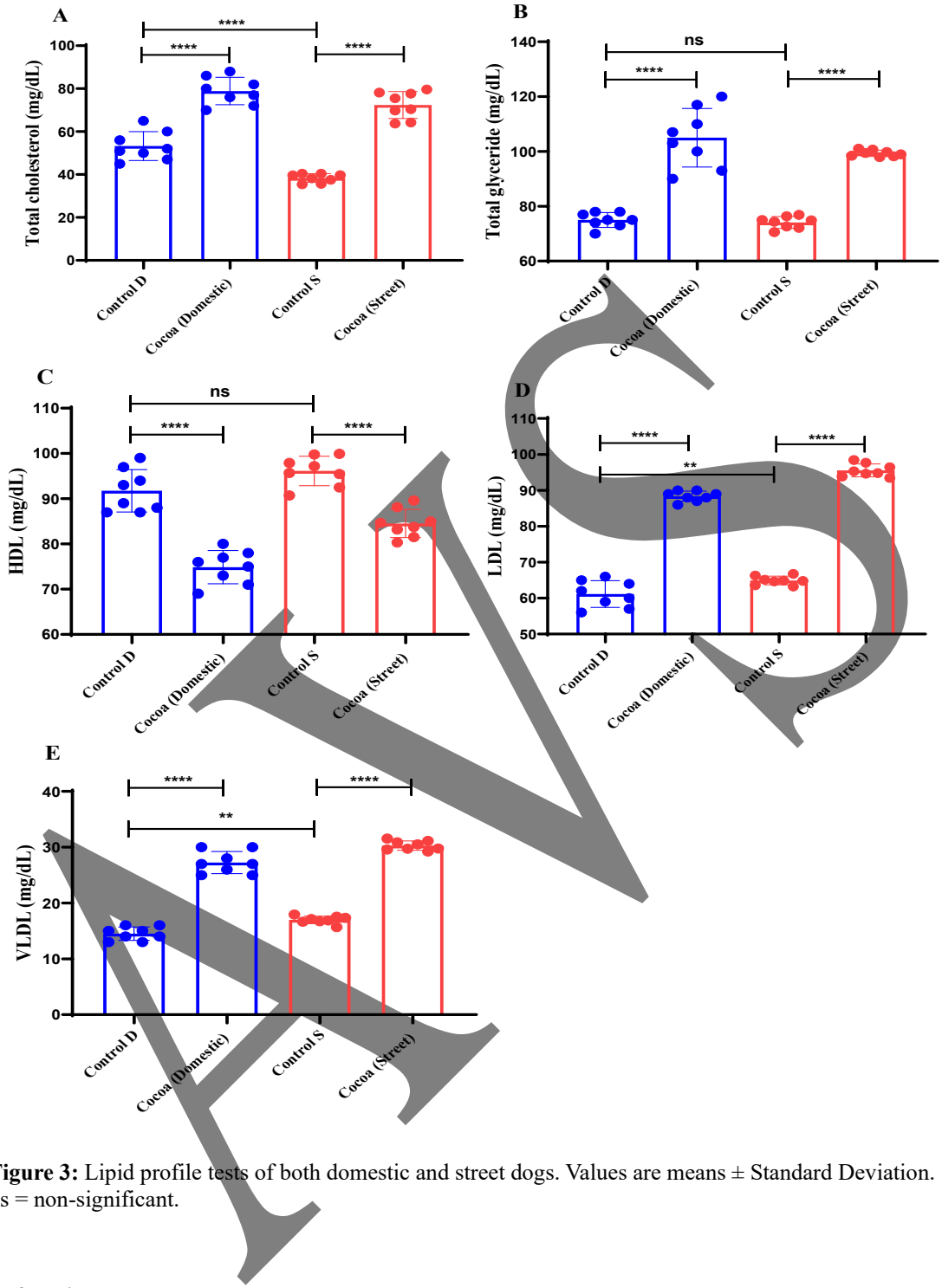

Figure 3: Lipid profile tests of both domestic and street dogs. Values are means ± Standard Deviation. ns = non-significant.

3.5. 3.5 Kidney function tests:

The effect of cocoa consumption on some kidney functions was investigated in the current study by measuring serum urea (mg/dL), serum creatinine (mg/dL), and total serum protein (g/dL). In Figure 4 A, there is a clear trend of increasing serum urea values (one-fold) in cocoa-treated groups of both domestic and street dogs compared with their control groups ($P<0.0001$). No significant differences were found between control D and control S.

Turning now to the experimental evidence on serum creatinine levels as can be seen from Figure 4 B the cocoa-treated groups of both domestic and street dogs reported significantly more than their controls ($P<0.0001$). There was no significant difference between the controls of domestic and street dogs.

In the final part of the kidney function test, cocoa led to a significant increase in the values of serum proteins in both domestic and street dogs ($P<0.0001$), however, there were no differences in their controls.

Looking at Figure 4, it is apparent that cocoa harms the kidney functions in both domestic and street dogs almost equally. RM one-way ANOVA was used to compare the groups, corrected with Sidak's multiple comparisons tests.

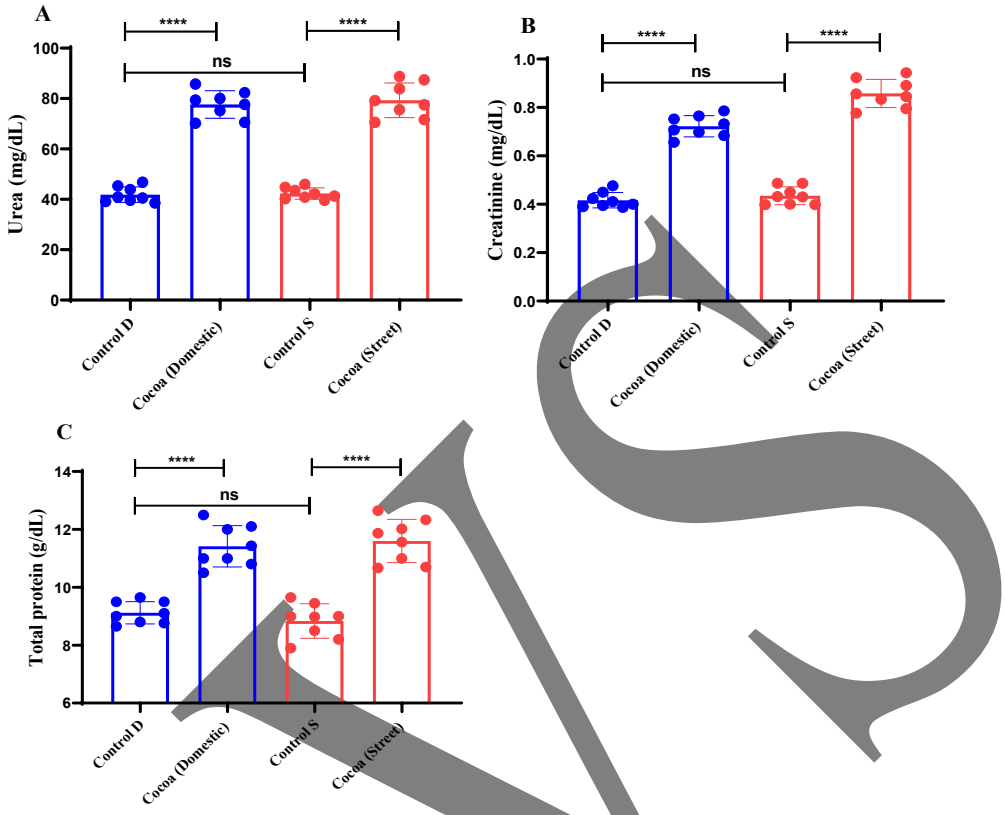

Figure 4: Kidney function tests of domestic and street dogs treated with cocoa compared with controls. Values are means ± Standard Deviation ns: is non-significant.

3.6. Histopathology:

3.6.1.Liver sections:

Figure 5 A (domestic dogs' group) and B (street dogs' group) both refer to several histopathological changes in the liver parenchyma including the loss of normal architecture, and cloudy and swollen hepatocytes. As shown in Figure 5 A, most cells have karyopyknotic nuclei, and others with dark, condensed nuclei, and diluted sinusoids with a clear increase in the size of Kupffer cells and infiltration of inflammatory cells. Looking at Figure 5 B, the hepatic portal vein is dilated and is surrounded by degenerated hepatocytes and inflammatory cells, loss of hepatocyte boundaries, dilution of the sinusoids, and irregular hepatocytes.

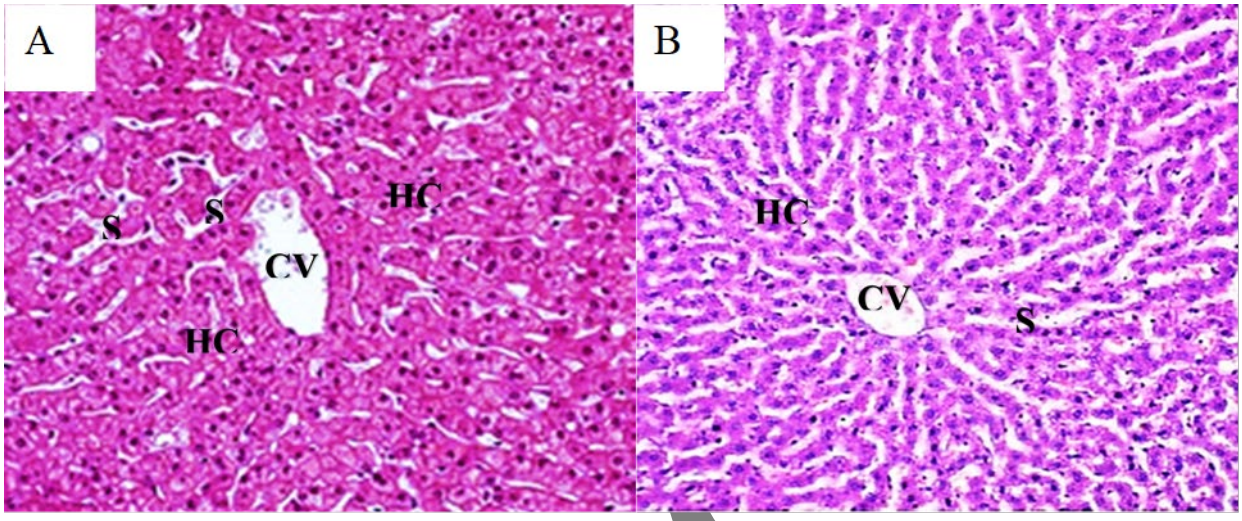

Figure 5: Liver sections of the control groups A) Domestic dogs and B) street dogs. Both figures show normal hepatocytes (HC), sinusoids (S), and clear central vein (CV). hepatocytes radiate as hepatic plates from a central vein (H&E 400X).

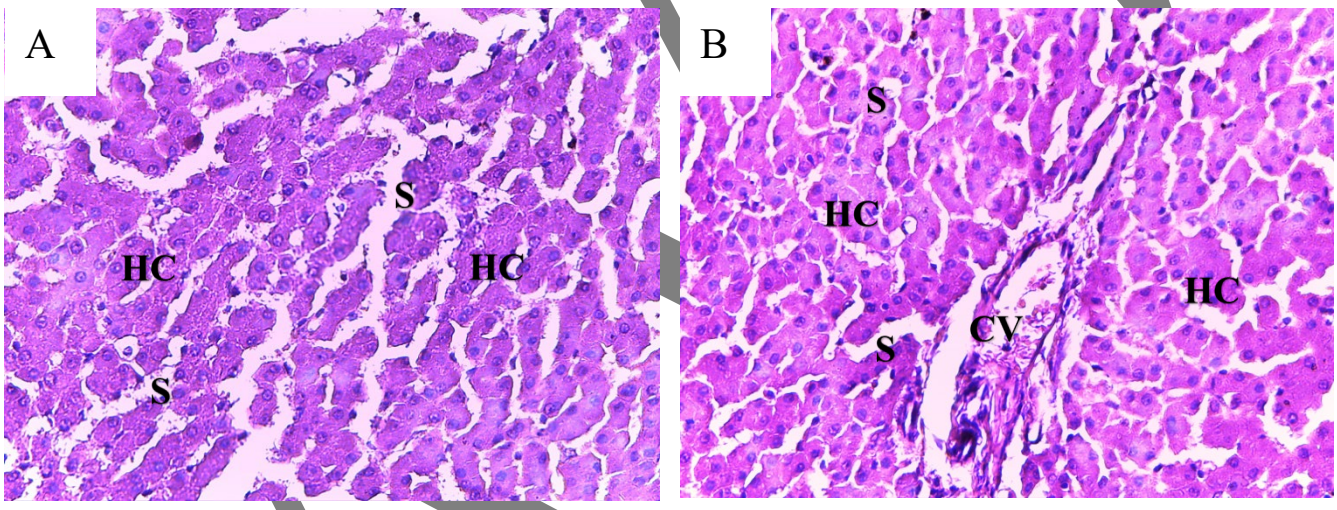

Figure 6: Histological sections of the liver of (A) domestic dogs and (B) street dogs both exposed to cocoa. There is clear congestion in the central vein (CV) and cloudy, swollen hepatocytes (HC) with obvious vacuolation and degeneration of sinusoids emptied into the central vein (H&E 400X).

3.6.2.Kidney sections

Histological sections in the kidneys of dogs of control groups show normal renal components as can be noticed in Fig. 7 (A) a sample from street dogs' group and (B) from domestic dogs' group. On the other hand, Fig. 8 (A) and (B) show the kidney sections of dogs exposed to cocoa from street and domestic groups respectively. There are variable changes including degeneration and necrosis in lining epithelial cells of renal tubules, sloughing of some cells from tubules epithelial region towards the cavity and most nuclei showed condensed and hypertrophied. In addition, there is a dilation in some renal capsules with lobulated glomeruli and others are completely atrophied with degenerated glomeruli.

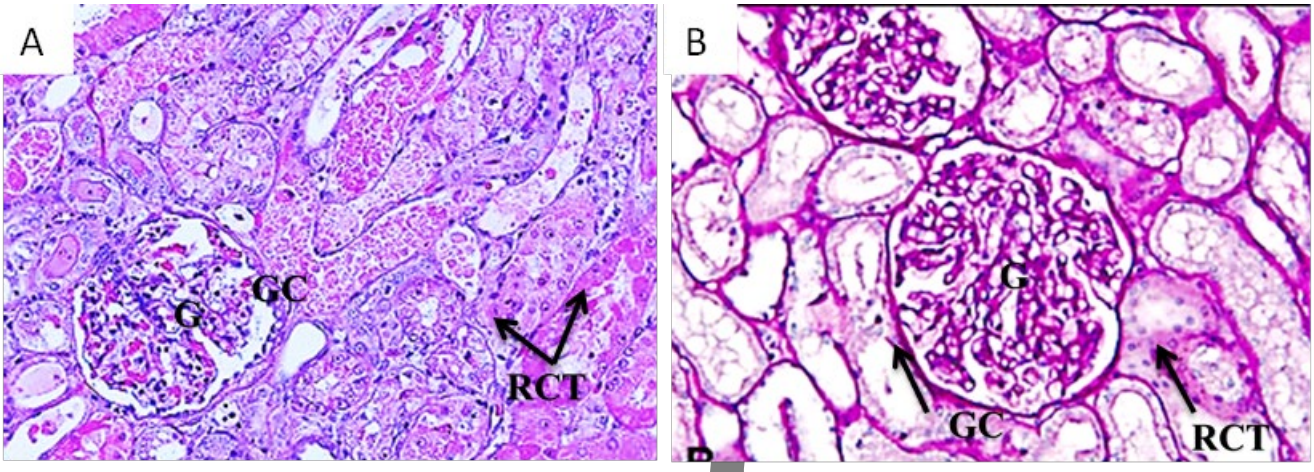

Figure 7: Kidney sections of the control groups A) Domestic dogs and B) Street dogs show normal renal glomeruli (G), normal glomerular capsule (GC), and normal renal convoluted tubules (RCT) lined with cuboidal epithelium (H&E 400X).

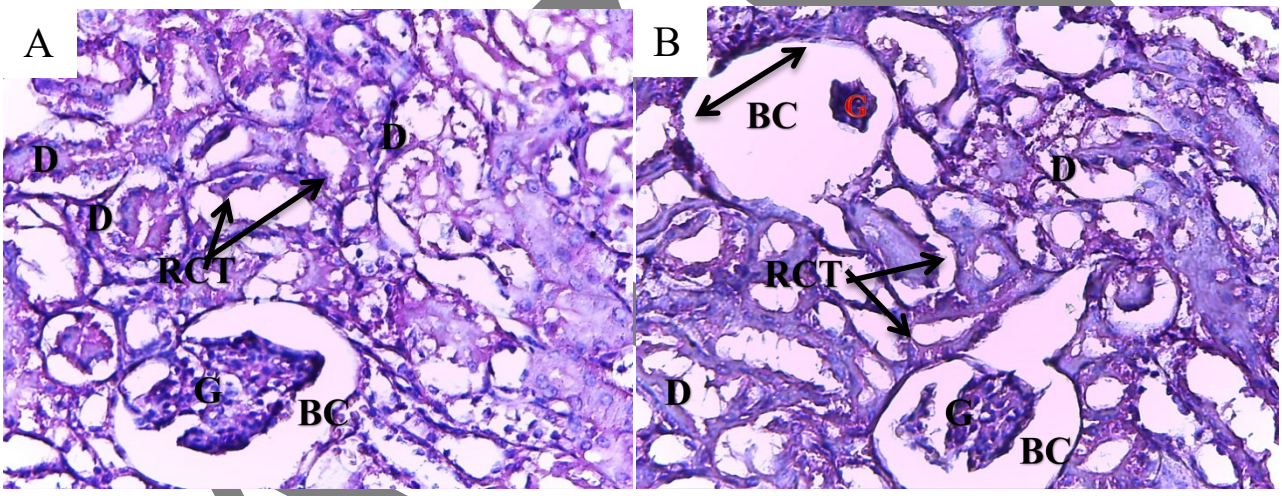

Figure 8: Kidney section of A) street dogs and B) domestic dogs treated with cocoa. Both figures show an atrophy of glomeruli (G) and an increase in the size of Bowman's capsule (BC). There is a degeneration in the renal tubules (D), and degeneration and vacuolation of renal convoluted tubules (RCT) (H&E 400X).

4. Discussion

Cocoa poisoning (chocolate toxicity) is considered an emergency toxic condition in dogs due to methylxanthine content such as theobromine and caffeine (Cortinovis & Caloni, 2016). Although both components contribute to the toxicodynamic; however, theobromine is considered to be the main contributor because its concentration in cocoa is about 3 to 10 times higher than caffeine and the half-life is significantly longer (Okiyama *et al.,* 2018).

The current study found that there was a delay in the response to toxic substances in street dogs in comparison to domestic dogs. This was noticed in the period of clinical signs initiation in domestic dogs (within the first 24 hours) compared with up to 72 hours in street dogs. A possible explanation for this might be that there were differences in the gastrointestinal tract condition resulting from the differences in lifestyles of these two groups. Different studies around the world focused on the differences in intestinal activity between street dogs and domestic dogs. They have suggested that street dogs are more likely to be infected with enterobacteria and helminths than domestic dogs which might lead to a change in the kinetics of many drugs and toxins (Erwanas *et al.,* 2014; Kalkofen, 1974; Miró *et al.,* 2007; Trasviña-Muñoz *et al.,* 2017). This can be due to the feeding nature between the two classes, where the domestic dogs receive a well-balanced hygienic diet, while the street dogs depend mainly on trash and unhygienic food.

Another possible explanation for the delay in response is resistance to the toxic substances due to previous exposure. As mentioned above, street dogs are more likely to be exposed to toxic materials including cocoa from their uncontrolled food in small quantities leading to multiple resistance. On the other hand, domestic dogs can be exposed to toxic materials such as cocoa in their

owners' houses. However, domestic dogs are more likely to be observed and obtain necessary medical assistance in case of toxicity, while survivor street dogs could tolerate the toxicity cases and develop resistance against different xenobiotics.

The early signs of toxicity that appeared in domestic dogs were vomiting, hematemesis, and polydipsia within the first few hours of ingestion. Other signs including panting, muscle twitching, and seizures, were come thereafter. These findings are consistent with previous studies that focused on canine behavior due to methylxanthine toxicity (Finlay & Guiton, 2005).

Prior studies that have noted the importance of cocoa toxicity found that the toxic substances can affect the respiratory, cardiovascular, and central nervous systems (Weingart *et al.,* 2021). There are several possible explanations for these results, Firstly the toxicokinetic of theobromine in dogs is easily absorbed from the gastrointestinal tract and widely distributed throughout the various organs of the body. However, it is slowly metabolized in the liver and undergoes enterohepatic recycling, with a half-life of 18 hours and it needs up to three days to be excreted in urine. This might lead to the accumulation of theobromine in the body and exert its toxic effect. This can also explain the rise in liver enzymes AST, ALT, and ALP which is consistent with the result of the current study as can be noticed in Figure 1.

Another possible explanation is that the toxic principles (theobromine and caffeine) of cocoa are competitively inhibiting adenosine receptors, resulting in tachycardia, CNS - stimulation, and diuresis (Stidworthy *et al.,* 1997). It also increases intracellular calcium levels by enhancing calcium influx and inhibiting intracellular calcium sequestration by the sarcoplasmic reticulum of the striated muscles. The net effect is to increase the strength and contractility of the skeletal and cardiac muscles. Methylxanthines may also compete for benzodiazepine receptors within the CNS and inhibit phosphodiesterase, resulting in increased levels of cyclic adenosine monophosphate (cyclic AMP), and also may increase circulating levels of epinephrine and norepinephrine (Gwaltney-Brant, 2021).

The effect of these compounds on kidney function and histology showed significant changes after ingestion of cocoa in both domestic and street dogs. Theobromine has a diuretic and smooth-muscle relaxation effect, and this effect is achieved by increasing glomerular filtration rate and inhibition of sodium and water reabsorption, which is more sustained than that of theophylline, but less pronounced (Gwaltney-Brant, 2021).

5. Conclusion

In conclusion, cocoa is toxic to both domestic and street dogs in almost the same manner. According to this study, the main difference is the starting of toxicity signs, where the domestic dogs responded faster than street dogs. This might be due to biological and or environmental factors. Moreover, there are other factors rather than lifestyle that can determine the severity of poisoning such as the size of animals, the type of chocolate (concentration of toxic principles), and finally the duration and the frequency of exposure.

Ethical approval:

The research related to animal use has complied with all the relevant national regulations and institutional policies for the care and use of animals. The protocol was approved by the Ethics Committee of the College of Veterinary Medicine at the University of Basrah (Project identification code: 38 on 7th December 2022). For further reference please contact the Committee at: wasfi.masoudi@uobasrah.edu.iq.

Conflict of interest:

The authors declare no conflict of interest.

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