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PROTECTIVE ROLE OF COLOSTRUM AGAINST CCL_4 INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

This study was carried out to evaluate the effect of cow and buffalo colostrum as a natural product on liver and kidney markers and antioxidant capcities after acute carbon tetrachloride (CCL_4) exposure in male Sprague-Dawley rats. Our results showed that the activities of liver and kidney markers were significantly ($P < 0.05$) increased while the activities of antioxidant enzymes were significantly decreased in treated rats with CCL_4. Oral administration of buffalo or cow colostrum for rats treated with CCL_4 resulted in a significant decrease in the activities of liver and kidney markers and a significant increase in the activities of antioxidant enzymes were observed. No significant difference between rats administrated with cow or buffalo colostrum in their values of liver and kidney markers and antioxidant activities. Our results suggest that both types of colostrum could protect liver and kidney damage through enhancement the antioxidant capacities and improvement liver and kidney functions.

KEYWORDS: Buffalo colostrum, Natural products, Hepatoprotective, Biochemical markers, Antioxidant capacity.

1. INTRODUCTION

Hepatitis and kidney failure are the major public health problem worldwide, responsible for considerable morbidity and mortality from chronic liver and kidney diseases.[1] Hepatitis C is one of several viruses in the hepatitis family causing liver inflamation and it cannot function properly and remove harmful material from the blood or convert food into energy. Worldwide, it is estimated that 130-170 million people are living with chronic hepatitis C infection, that it infects 3-4 million people per year, and that more than 350,000 people die

from hepatitis C related diseases each year. Countries with particularly high rates of infection include Egypt (22%), Pakistan (4.8%) and China (3.2%).[2]

Carbon tetrachloride (CCL_4) has been used extensively one of chemical compounds in animal model system, with which it is studied liver hepatitis induced by free radicals in rats and mice. Liver damage caused by CCL_4 is regarded to inflammation in early stage as the analogue of hepatotoxins in humans. In the principle of liver damage, CCL_4 is reductively bioactivated by cytochrome P450 2E1into a trichloromethyl radical ($^{\cdot}OOCCl_3$), which is subsequently converted into peroxyl radical in the presence of oxygen. These reactive free radical metabolites can covalently bind to macromolecules and also initiate lipid peroxidation.[3] Antioxidative action plays an important role in protection against CCL_4 induced liver injury. Protective effects of various natural products in CCL_4 hepatotoxicity have been reported.[4]

Colostrum is an amazing material that like many other things in nature, reflects the evolutionary development of a unique composition that will serve the needs of the offspring for which it is intended. Colostrum is the first milk produced by mammals after birth as the first six postpartum (3-4 days) and is particularly rich in immunoglobulin, antimicrobial peptides (e.g., lactoferrin, and lactoperoxidase) and other bioactive molecules including growth factor, plus anti-inflammatory, antioxidant and immune enhancing components.[5,6] Colostrum had higher concentrations of caseins and whey proteins than mature milk and there is evidence that caseins and whey proteins from colostrum exert antioxidant activities measured by reducing power, ferrous ion chelating abilities as well as inhibitory effects on lipid peroxidation.[7] Different antioxidative enzymes in colostrum can prevent the formation of radicals or scavenge radicals or hydrogen peroxide and other peroxides. Among these enzymes, lactoperoxidase, catalase, superoxide dismutase and glutathione peroxidase. Non-enzymatic antioxidants in colostrum act as radical scavengers, such as lactoferrin, Vit. A and E, carotenoids and flavonoids.[8]

There are not enough studies on the protective effect of buffalo colostrum against liver damage. Therefore, our study aimed to evaluate the hepatoprotective effect and antioxidative activities of cow and buffalo colostrum, the two main types of colosrum found in Egyptian markets, against CCL_4 induced liver damage in rat.

2. MATERIALS AND METHODS

2.1. Materials

Fresh cow and buffalo colostrum were collected after two hours of parturition from the herd of the Faculty of Agriculture, Cairo University, Giza, Egypt.

2.1.2. Chemicals

Gum acacia (5%), carbon tetra chloride (CCL_4), starch, cellulose, casein, vitamins and salt mixture were obtained from EL-Nasr pharmaceutical chemical Co., Cairo, Egypt. Corn oil was purchased from the local supermarket.

Kits for determination of aspartate and alanine aminotransferase (ALT and AST), alkaline phosphatase (ALP), serum gamma glutamyl transferase (GGT), superoxide dismutase (SOD), catalase (CAT), glutathion-S-transferase (GST), Glutathion peroxidase (GPx), urea, creatinine, total bilirubin and direct bilirubin were obtained from Biodiagonstic company, Giza, Egypt.

2.1.3. Animals

Male Sprague-Dawley (SD) rats weighting 180 ± 10 g were procured from animal house Lab., Agricultural Research Center, Giza, Egypt. This study was approved by the Committee of Scientific Ethics at Agricultural Research Center, Giza, Egypt and according to its guidelines.

2.2. Experimental protocol

2.2.1. Basel diet preparation

Basal diet was prepared according to Kawasaki et al. (2009).[10] The chemical composition of the diet was as follows, casein (17%) starch (70%), soy bean oil (3%), cellulose (4.5%), salt mixture (4%) and vitamin mixture (1%).

2.2.2 Lyophilized colostrum preparation

Cow and buffalo colostrum samples were freeze – dried using freeze –drying (LY-5M-R133 inijders scientific, Amsterdam, Holland). Lyophilized cow and buffalo colostrum were suspended in 5 % gum acacia for rats oral administration.

2.2.3. Animal groups

All SD rats were fed basal diet and water for one week as adaption period and divided into four groups (G), each group contains eight rats.

G1: rats were fed basal diet.

G2: rats were injected with CCL_4 after 2 weeks of fedding basal diet.

G3: rats were injected with CCL_4 after 2 weeks of oral administration of cow colostrum and fedding basal diet.

G4: rats were injected with CCL_4 after 2 weeks of oral administration of buffalo colostrum and fedding basal diet.

The experiment period was 4 weeks. Daily colostrum dose for each rat was 0.1g/ 100g body weight as a recommended therapeutic dose of colostrum for human[11] and[12] in mice model.

2.2.4. Hepatitis induction

Rats were injected intraperitoneally by 50% CCL_4 (v/v) in liquid paraffin. Rats were treated with CCL_4 at dose of 0.5 mL/ kg body weight to induce hepatic injury.[13]

2.2.5. Blood samples collection

After 4 weeks, animals were anesthetized with ether and blood samples were collected with or without anticoagulant (10 mM EDTA) from retro- orbital venus plexus. Blood samples of all treatments were centrifuged at 3000 rpm/20 min. separated plasma or serum was stored in Ependrop vials in freezer at -18 °C till the biochemical analysis.

2.3. Methods of Analysis

2.3.1. Chemical and microbiological analysis of colostrum

Cow and buffalo colostrum were examined chemically according to A.P.H.A (1992)[14] and microbiologically according to Oxoid (1982).[15] The obtained data are shown in Table (1).

2.3.2. Biochemical assay

The activities of AST, ALT, ALP, GGT, SOD, CAT, GST, GPx, urea, creatinine, total bilirubin and direct bilirubin were measured using the colorimetric methods described in the kits from Biodiagonstic, Giza, Egypt and according to the clinical guide to laboratory tests.[16]

2.3.3 Statistical analysis

Results were analyzed statistically with SPSS 10.0 one – way ANOVA to assess the significance of differences between groups with $P < 0.05$ being considered significant. Data were expressed as mean ± standard error (SE). The least significant difference (LSD) and one way analysis of variance were used for multiple comparisons between treatments. Each test was analyzed out in triplicate.[17]

3. RESULTS AND DISCUSSION

3.1. Effect of oral administration of cow and buffalo colostrum on liver and kidney weight in CCl_4 treated rats.

The variations in the liver and kidney weights of rats subjected to difference treatments are shown in Figure (1). During the course of present investigations, it was observed that liver and kidney weights were significantly ($P < 0.05$) decreased in rats group injected with CCL_4 (G2) in comparison with rats in control group (G1). Also, the data obtained in the same Figure showed that oral administration of either cow (G3) or buffalo (G4) colostrum had significant protection of liver and kidney from weight loses induced by CCL_4 in comparison with G2. Furthermore, no significant differences in liver and kidney weight values could be observed between rats orally administrated with cow or buffalo colostrum. These results are in agreement with those reported by Ko et al (2006)[13] who reported that CCL_4 resulted in a significant decrease in the body and liver weights of mice. On the other hand, the shrink effect of CCL_4 on liver and kidney weight was significantly decreased in rats of groups 3 and 4. Colostrum could enhance kidney function and regrowth kidney or liver tissues that ordinarily shrink with age or injury[21] and protein synthesis and body weight in pigs.[22]

3.2. Effect of oral administration of cow and buffalo colostrum on liver markers and plasma antioxidant enzymes activity.

The obtained data in Table (2) reveal that different liver markers including ALT, AST, ALP, GGT, total bilirobin and direct bilirobin are significantly increased in the CCL_4 treated rats (G2) in comparison with rats in G1, G3 and G4. Also, no significant difference could be found between rats administrated orally with cow (G3) or buffalo (G4) colostrum in their liver markers. Data in Table (3) show that oral administration of cow or buffalo colostrum can promote the plasma antioxidant enzymes in rats of G3 and G4 as compared with rats in G1 and G2.

Carbon tetrachloride (CCL_4) is used in production of toxic free radicals (CCL_3) which causes injury for different tissuses including brain, liver, kidney and skin[3] resulting in an increase in levels of liver markers[19] and levels of direct bilirubin.[20] Our results indicate degenerative changes and hypofunction of the liver and showed that the harmful and hepatotoxicity effect of CCL_4 that administrated in rats or mice.[23,24] Also, the hepatoprotective effect of colostrum may be attributed to the colostrum contains high amounts of immunoglobulin A (IgA)[9] and whey proteins[35,36] that have protective effects against CCL_4 damage.

Antioxidant enzymes (SOD, CAT, GST, and GP_x) play an important role in liver protection against CCL_4 induced liver injury. SOD plays an important role in the elimination of reactive oxygen species derived from the peroxidative process in liver tissues.[25] Moreover, SOD removes superoxide by converting it to hydrogen peroxide which can be rapidly converted to water by catalase.[23] Treatment with CCL_4 (G2) in Table (3) shows a significant decrease ($P < 0.05$) in all plasma antioxidants parameters values that might be due to the principle causes of CCL_4 induced hepatic damage are lipid peroxidation, decrease of antioxidant enzymes activities and generation free radicals.[26] On the other hand, treatments with administration cow or buffalo colostrum (G3 and G4, respectively) showed a significant ($P < 0.05$) increase in all plasma antioxidants parameters. The ability of colostrum to promote plasma antioxidant enzymes was due to that colostrum itself can be the source of reactive oxygen species (ROS) due to the presence of macromolecules susceptible to peroxidative damage as well as ROS generating systems.[27] Also, human experiments showed that oxygen radical absorbing capacity was high in colostrum.[28]

3.3. Effect of oral administration of cow and buffalo colostrum on serum kidney markers in CCL_4 treated rats.

Results in Figure (2) illustrate that CCL_4 injection caused a significant ($P < 0.05$) increase of serum creatinine and urea (renal failure markers) values in G2 in comparison with G1. On the other hand, oral administration of cow or buffalo colostrum (G3 and G4, respectively) could significantly reduce serum creatinine and urea values in rats treated with CCL_4 in comparison with rats in G2. Chronic kidney disease (CKD) is identified by a blood tests for creatinin and urea levels. High levels of creatinin and urea indicate a falling glomerular filtration rate and follwed by a decrease in the capability of the kidneys to excrete waste products. Free radicals formed from CCL_4 metabolism induce lipid peroxidation and believed to be one of the major causes of cell membrane damage leading to a number of pathological situations by causing acute and chronic renal injuries in animal models[29,30] and humans.[31] Treatment with CCL_4 (G1) as can be observed in Figure (2) shows significant ($P < 0.05$) increase in urea and creatinin parameters as a result of kidney tissues damage.[32] On the other hand, oral administration with either cow or buffalo colostrum could decrease levels of creatinin and urea in rats injected with CCL_4 (G3 and 4, respectively). Results reported by Davis et al (2007)[33] illustrated that bovine colostrum didn't have any toxic effect on liver or kidney functions in rats.

Table 1. Chemical composition (%) and microbiological analysis (log cfu mL^{-1}) of colostrum samples.

Colostrum Samples	Chemical Composition %					Microbiological analysis Log cfu mL^{-1}			
	Total Solids	Fat	Protein	Lactose	Ash	Total count	Mold & Yeast	Coliform	*Staph. aureus*
Cow	19.90± 0.50	5.15± 0.30	12.51±0.18	1.60±0.30	0.72±0.13	3.30±0.23	1.50±0.41	ND	ND
Buffalo	28.70± 0.25	10.00±0.42	14.00±0.15	2.62±0.26	1.88±0.16	3.40±0.35	1.30±0.52	ND	ND

±: Standard error

Table 2. Effect of oral administration of cow or buffalo colostrum on some liver functions in CCL_4 treated rats.

Treatments*	ALT**	AST**	ALP**	GGT**	Total Bilirobin	Direct Bilirobin
	UL^{-1}				$mg\ dL^{-1}$	
G1	186.00 ± 0.70^{b}	355.77 ± 0.60^{b}	128.73 ± 0.60^{b}	5.06 ± 0.50^{b}	16.12 ± 0.60^{b}	12.13 ± 0.70^{b}
G2	210.76 ± 2.70^{a}	389.83 ± 0.80^{a}	149.80 ± 1.05^{a}	12.10 ± 2.60^{a}	27.90 ± 1.60^{a}	23.55 ± 2.80^{a}
G3	186.50 ± 1.50^{b}	362.50 ± 1.30^{b}	139.56 ± 1.70^{b}	6.20 ± 1.05^{b}	20.32 ± 2.02^{b}	17.53 ± 0.90^{b}
G4	184.60 ± 2.60^{b}	359.96 ± 1.50^{b}	130.60 ± 2.05^{b}	5.26 ± 1.70^{b}	20.90 ± 0.70^{b}	17.64 ± 2.60^{b}

*: G1: rats were fed basal diet, G2: rats injected with CCL_4 after 2 weeks of feeding basal diet, G3: rats injected with CCL_4 after 2 weeks of oral administration of cow colostrum, G4: rats injected with CCL_4 after 2 weeks of oral administration of buffalo colostrum.

**: ALT: Aspartate aminotransferase, AST: Alanine aminotransferase, ALP: alkaline phosphatase, GGT: serum gamma glutamyl transferase.

±: Standard error.’

Table 3: Effect of oral administration of cow or buffalo colostrum on antioxidant activity (UL^{-1}) in CCL_4 treated rats.

Treatments*	SOD**	CAT**	GST**	GP_x**	GSH**
G1	30.34 ± 1.02^a	29.64 ± 2.70^a	33.48 ± 1.60^a	36.69 ± 0.80^a	33.04 ± 1.90^a
G2	17.32 ± 2.70^b	13.76 ± 1.80^b	15.22 ± 2.80^b	12.19 ± 2.90^b	19.46 ± 2.70^b
G3	34.58 ± 1.45^a	29.38 ± 1.50^a	35.02 ± 1.70^a	33.34 ± 0.56^a	33.40 ± 1.68^a
G4	39.94 ± 1.90^a	36.46 ± 2.60^a	37.78 ± 0.90^a	34.63 ± 1.00^a	43.30 ± 1.80^a

*: G1: rats were fed basal diet, G2: rats injected with CCL_4 after 2 weeks of feeding basal diet, G3: rats injected with CCL_4 after 2 weeks of oral administration of cow colostrum, G4: rats injected with CCL_4 after 2 weeks of oral administration of buffalo colostrum.

**: SOD: Superoxide dismutase, CAT: Catalase, GST: glutathion-S-transferase, GPx: Glutathion peroxidase and GSH: reduced glutathione.

±: Standard error

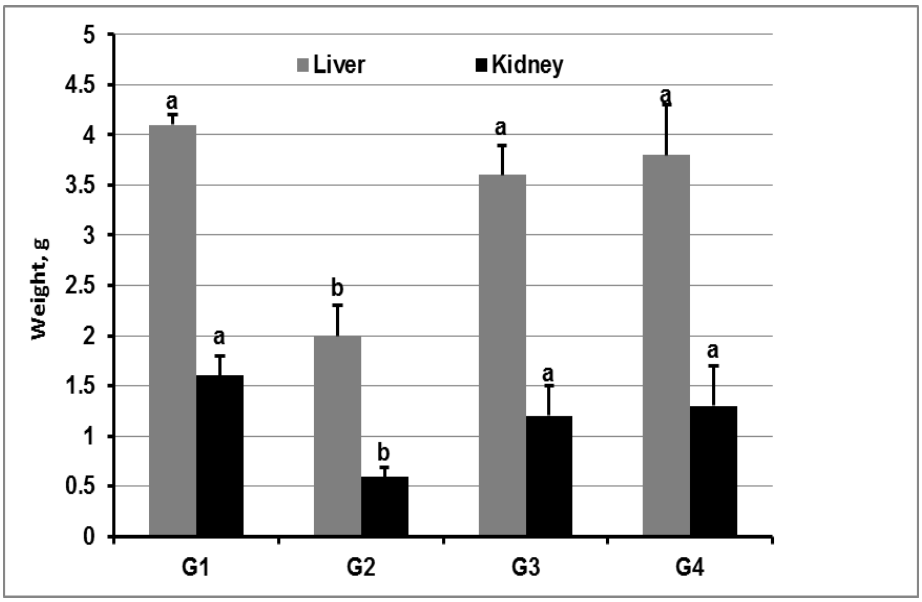

Figure 1: Effect of oral administration of cow or buffalo colostrum on liver and kidney weight (g) in treated CCL_4 rats.

G1: rats were fed basal diet, G2: rats injected with CCL_4 after 2 weeks of feeding basal diet, G3: rats injected with CCL_4 after 2 weeks of oral administration of cow colostrum, G4: rats injected with CCL_4 after 2 weeks of oral administration of buffalo colostrum.

Error bars represent means of three replicates.

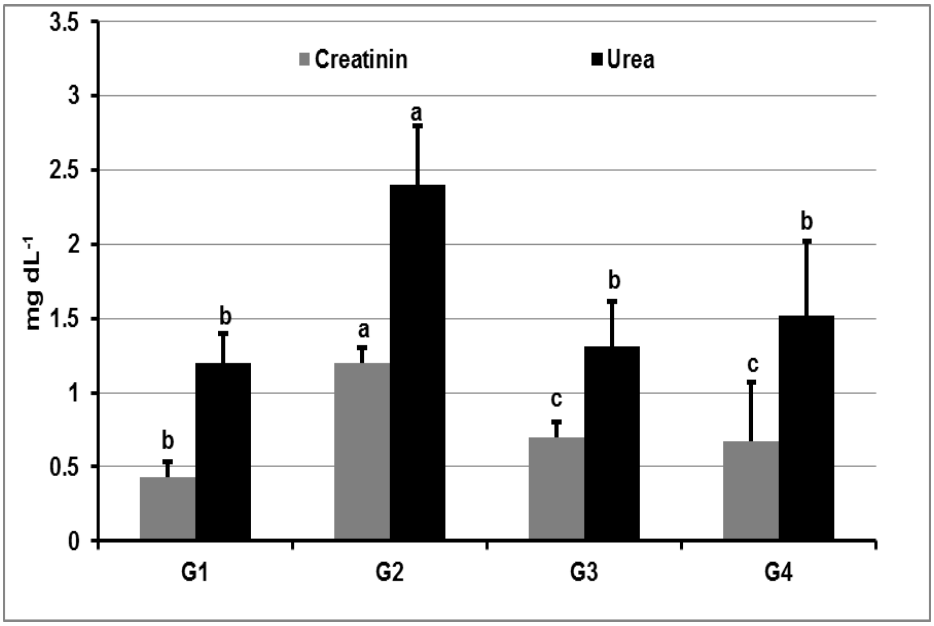

Figure 2: Effect of cow and buffalo colostrum administration on the concentration of serum creatinin and urea ($mgdL^{-1}$) in treated CCl_4 rats.

G1: rats were fed basal diet, G2: rats injected with CCL_4 after 2 weeks of feeding basal diet, G3: rats injected with CCL_4 after 2 weeks of oral administration of cow colostrum, G4: rats injected with CCL_4 after 2 weeks of oral administration of buffalo colostrum.

Error bars represent means of three replicates.

4. CONCLUSION

Colostrum is a natural product and it is rich source in bioactive components. Cow and buffalo colostrum are the major two types of colostrum in Egyptian markets. Our findings indicated that oral administration of either cow or buffalo colostrum could enhance the liver and kidney weights, liver and kidney functions and antioxidant activities after CCL_4 exposure. Therefore, both types of colostrum might be used as a dietary streatgy for enhancing of liver functions and antioxidant capacities. Indeed, it would be interesting to investigate the molecular mechanisms by which both main types of colostrum in Egypt can protect liver and kidney from damage induced by CCL_4.

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