# Possible protective effect of Costus root extract on liver of rat prior exposure with melamine: The hematology, biochemistry, histopathology and ultrastructure study

**Khalid Hassan** ( kebraheem@tu.edu.sa )
Taif University Faculty of Medicine

**Research Article**

**Keywords:** Costus root extract, melamine, liver, histopathology, albino rats

**Posted Date:** May 18th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1552127/v1

# Abstract

The current study evaluated the protective effect of Costus root extract against the hematology, biochemistry, histopathology and ultra-structure changes induced in rat liver due to the sub chronic exposure with melamine. In recent years manufacturers adding melamine (MA) to milk to increase its protein content but this led to toxic effects on many organs such as kidneys, liver, brain, etc. in experimental animals. Recent studies have proved that Costus root extract has a hepatoprotective role and has many health-related benefits showed against various toxins related to oxidative stress. Eighty adult albino rats (200–250 gm) were further allocated into 04 equal sets; set 01 exposed with distilled water orally, set 02 exposed with costus plant extract (0.4 mg/ kg) orally, set 03 exposed with MA (50mg/kg b.wt/day), and set 04 exposed with MA (50 mg/kg b.wt/day) plus costus plant extract (0.4 mg/ kg) orally for the period of two months. The experimental rats were exposed to general anesthesia before the sacrifice and the sample of blood and liver were collected. The samples were further prepared for histopathological and ultrastructural examinations. The results revealed that MA treatment (set 03) caused hematological, biochemical, histopathological, and ultrastructural alterations. Co-administration of Costus root extract with MA ameliorated most of the toxic effects of melamine (set 04). The results demonstrated that Costus root extract exhibited a protective effect by decreasing the toxic pathological changes caused by MA in the liver of albino rats.


# 1. Introduction

The liver is one of the important organs responsible for the metabolic and excretion based activities of the human body. It implements a most important function in a remove toxic substances and xenobiotics. Recently liver diseases were considered as most common cause of mortality and morbidity over the world mainly due to dysfunction in removing of toxic chemicals (AlSaadi et al. 2018). Melamine (MA) is an organic compound known for the synthesis from a nitrogen based compound regarded as urea. Urea contains 66% of nitrogen, during the synthesis of melamine obtained cyanic acid. In industry combination of melamine with formaldehyde gave melamine resin that used in durable thermosetting plastic, flame retardants, cloths, adhesive substances, dry erase boards, and housewares(Chain et al. 2010). Yellow pigment which is used as a dye, in plastics and inks is produced from MA. Arsenic drugs are used for the treatment of African trypanosomiasis contained also derivatives of MA (Hau et al. 2009). In recent years, last researchers found that ingestion of MA-tainted food caused large number of accidental deaths of domestic animals (Dorne et al. 2013, Rumbeiha &Morrison 2011). In addition to that they noted that many cases consumption of MA-contaminated infant formula in various countries lead to MA poisoning they also concluded that effects of MA consumption might lead to toxicity to reproductive systems, kidney inflammation, cancer bladder, nerve destruction also researchers found those children who ingested powdered infant formula contaminated with MA- showed symptoms of immune-compromised disorders additionally MA-cyanurate caused aggregation and erythrocyte deformation (Dobson et al. 2008, Ogaly et al. 2015, Ogasawara et al. 1995, Organization 2009, Wang et al. 2010, Yin et al. 2016, Yin et al. 2014, Yin et al. 2013, Zhou et al. 2010). It was also reported that the MA administered

at a dose of 50 mg/kg/day for further consecutive days caused cytotoxic effects on lymphocytes of spleen (Yin et al. 2014). Herbal Plant extracts had been showed recently a natural protective agent against various toxins (Organji et al. 2015).

The Costus specious is an Indian medicinal plant related to family Coastaceae (Zingiberaceae), usually used in the treatment of different diseases (Lijuan et al. 2011). Several studies reported that different extracts of plant that possesses antioxidant potential which prevent against MA hepatotoxicity. It promotes the antioxidant activity of enzyme and suppressed the peroxidation of lipid.

The Costaceae (Zingiberaceae) family comprises into 52 genera and about 1,300 species presented in many countries like Asia, tropical Africa, and America (Lijuan et al. 2011). Costus specious has many classical uses like expectorant, tonic, purgative, astringent, stimulant, and anthelmintic (Gupta 2010). Roots of Costus specious have hepatoprotective action, anti-fungal, antibacterial, antioxidant, analgesic, anti-inflammatory, anti-hyperglycemic, antipyretic and anti-hyperlipidemia (Bavarva &Narasimhacharya 2008, Bhuyan &Zaman 2008, Ibrahim et al. 2019, Srivastava et al. 2013).

## 2. Materials And Methods

## 2.1. Costus extraction:

Preparation of Costus extract by the addition of 10 grams of powdered roots of Costus in 100 ml of boiled water for 3 to 5 minutes. Covered the decoction and kept at temperature less than 25°C (Sanna et al. 2012), then extract was given to rats by gastric tube.

## 2.2. Melamine preparation:

Melamine was obtained from commercial source (Sigma-Aldrich M2659). Melamine was diluted in distilled water for the preparation of working stock concentrations, which further used for the conductance of the experiment (Salem et al. 2018).

## 2.3. Experimental Animals:

The present study was done by preparing 80 adult, young and healthy albino rats belong from both sexes. The average body mass of the rats in the range of 220 to 250 grams, were obtained from Medical Research of the Taif University.

## 2.4. Animal groups:

These experimental rats were further divided into 04 equal sets:

Set 01 obtained normal distilled water orally,

Set 02 obtained treatment with costus plant extract (0.4 mg/ kg) orally (Sanna et al. 2012).

Set 03 obtained MA (50 mg/ kg body weight/ day), (Salem et al. 2018).

Set 04 obtained MA (50 mg/ kg body weight / day) along with costus extract (0.4 mg/ kg) orally for time period 2 months.

Experimental animals were sacrificed under general anesthesia then blood and liver were sampled and prepared for histopathology and ultra-structural examinations. All samples stained for histological and ultra-structural sections were carried out in laboratories of Taif University. All the experimental animal trials were proceeding as per the international laws and policies under the Ethics of Committee of Animal Experiments. The rats were kept in the room for fifteen days to adapt to the above-mentioned conditions and supplied with standard diet and water.

## 2.5. Blood samples:

After twenty-four hours of the end of the experimental period, the animals were rapidly decapitated after anesthetized Collection of blood then divided into two samples. The first sample collected in (EDTA) tube (heparinized tube) for the examination of hematological characteristics, and the second sample was let for clotting at 37°C. The sample was further centrifuged in order to collection of serum at the speed of 3000 rpm for 15 minutes. Serum was further pooled and kept at -20°C. Evaluate the activities of transaminase alanine amino transferase (ALT) and aspartate amino transferase (AST), alkaline phosphatase, total protein, albumin and total bilirubin from serum.

## 2.6. Tissue sample:

Small slices of liver rates were washed with normal saline after excision then divided into three sets, the first set was used for histological examination the second set was kept at -80°C and used for assessment antioxidant enzyme activity and oxidative stress the last set was processed for ultra-structure studies

## 2.7. Hematological studies:

Determination of blood cells by using heparinized blood sample collected in EDTA tubes, quantify red blood cells (RBCs), quantify white blood cells (WBCs), and the amount of hemoglobin (Hb%). Differential count was also performed for polymorph cells and lymphocytes cells with an Animal Blood Counter known as ABC vet.

## 2.8. Bio-chemical Characterization

Liver biomarker analysis

Evaluate measurement of alanine amino transferase (ALT) and aspartate amino transferase (AST) and these were estimated according to the method described in (Schumann &Klauke 2003). It was estimated by using reagent kits obtained from “Human Diagnostics” (Germany). The Serum albumin was estimated by using a commercial kit and was assayed as per method in (Schmidt &Eisenburg 1975).

## 2.9. Liver samples

Determination of oxidative stress of enzyme and antioxidant enzyme activity

Determination of catalase enzyme (CAT) and glutathione (GSH), content of malondialdehyde (MDA) and superoxide dismutase (SOD) (Bergmeyer et al. 1983, Ellman 1959, Kumar et al. 2012, Ohkawa et al. 1979).

# 2.10. Histological studies:

Light microscopy:

The liver tissue was stationary in buffered formalin solution (10%) for 24 hour for histological studies. It was further embedded in paraffin wax which has 5 mm thickness. The sections were stained with hematoxylin and eosin for histopathology (Bancroft &Gamble 2008).

### 2.11. Electron microscopy:

The liver samples were tearing into small fragments of 1 mm thickness. It was further static in phosphate buffer (pH 7.2) solution for the period of 3 hour at 4°C for electron microscopy. It was further suspended in epon-araldite solution. It was in the form of beam capsules which was labeled. The section of 50 n.m thickness were taken and considered as ultra-thick sections. These were further placed on copper-mesh gridirons, stained using uranyl acetate solution for 30 minutes. The sample was further exposed to lead citrate for 30 minutes (Bancroft &Gamble 2008).

### 2.12. Statistical analyses

All expressed data were statistically analyzed as the mean ± standard deviation (SD). Significance is given as *=$p < 0.05$, **= $p < 0.01$., using a one-way analysis of variance test. Further comparisons among groups were conducted according to Tukey's post hoc test using the Statistical Package for the Social Sciences (SPSS) version 18.

# 3. Results

# 3.1. Hematological findings:

Data in Table 1of rats of set 03 revealed significant decrease in RBC count, Hemoglobin level, total WBC count and lymphocyte but neutrophils and monocytes were significantly elevated in comparison to sets 01 and 02, on the contrary rats in set 04 showed significant increase in RBC count, hemoglobin level, total WBC count and lymphocyte, neutrophils and monocytes were significantly decreased in comparison to set 03.

Table 1
The Effect of MA with or without Costus extract on blood counts of rat

| Parameters | Set 01 (control) | Set 02 (Costus) | Set 03 (MA) | Set 04 (Costus + MA) |
|---|---|---|---|---|
| RBCS (x 106/µL) | 6.01 ± 0.81 | 6.05 ± 0.73 | 3.97 ± 0.82* | 6.08 ± 0.21** |
| Hb (g/dl) | 12.01 ± 0.03 | 11.92 ± 0.03 | 8.81 ± 0.72* | 11.09 ± 0.05** |
| HCT (%) | 51.01 ± 0.24 | 49.98 ± 0.26 | 42.31 ± 0.33* | 52.02 ± 0.49** |
| WBCS (x103/ ul) | 5.12 ± 0.38 | 5.18 ± 0.23 | 3.77 ± 0.37* | 5.02 ± 0.30** |
| Lymphocytes (%) | 32.14 ± 0.61 | 33.20 ± 0.51 | 26.44 ± 0.69* | 32.32 ± 0.56** |
| Eosinophils (%) | 0.02 ± 0.02 | 0.01 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.02 |
| Basophils (%) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Neutrophils (%) | 51.96 ± 0.53 | 52.05 ± 0.51 | 62.41 ± 0.79* | 53.97 ± 0.44** |
| Monocytes (%) | 3.31 ± 0.21 | 3.43 ± 0.21 | 8.53 ± 0.23* | .31 ± 0.35** |

Number (n) of participants per group = 20, SD = standard deviation, Set 01 (control) obtained equal amount of distilled water/day for 2 months, Set 02 will be obtained 1.5% w/v Costus plant extract (0.4mg/ kg) by mouth for the duration of two months, Set 03 obtained MA (50mg/kg body weight /day) for the period of two months, Set 04 obtained MA (50mg/kg body weight /day) plus Costus plant extract (0.4mg/ kg) by mouth for 2 months. * = $p < 0.05$, ** = $p < 0.01$.

# 3.2. Liver biomarkers:

Data in Table 2 of rats of Set 03 revealed significant increase in the level of AST, ALT and TSB in serum levels of while significant reduction in serum albumin as compared with Set 01 and 02, on the contrary rats in Set 04 showed significant reduction in the level of AST, ALT and TSB in serum. It was also observed that significant increase in serum albumin than Set 03.

Table.2: Effect of melamine used alone or with Costus extract on Mean + SD of liver functions of rat.

| Parameters | Set 01 (control) | Set 02 (Costus) | Set 03 (MA) | Set 04 (Costus+ MA) |
|---|---|---|---|---|
| AST (U /L) | 53.85±0.66 | 54.14±0.66 | 75.01 ± 0.21 * | 53.01±0.17** |
| ALT (U /L) | 91.14±0.76 | 91.04±0.76 | 144.92 ± 0.82 * | 92.01±0.04** |
| TSB (mg /dl) | 1.12±0.01 | 1.13±0.01 | 2.91 ± 0.01 * | 1.07±0.01 ** |
| Albumin (mg /dl) | 5.03±0.01 | 5.09±0.01 | 2.34 ± 0.02 * | 5.83±0.01 ** |

Number (n) per group = 20, SD = standard deviation, Set. 01 (control) obtained equal volume of distilled water/day for 2 months, Set. 02 will be obtained 1.5 % w/v Costus plant extract (0.4mg/ kg) by mouth for 2 months, Set. 03 obtained MA (50mg/kg body weight /day) for 2 months, Set 04 obtained MA (50mg/kg

body weight /day) plus Costus plant extract (0.4mg/ kg) by mouth for the duration of two months. * = p < 0.05, ** = p < 0.01.

## 3.3. Antioxidants-Lipid peroxidation:

Data in Table 3 revealed that rats of set 03 showed significant reduction in the level of enzyme including SOD, CAT and GSH. It was observed that significant enhance in MDA level as compared to sets 01 and 02. Meanwhile, rats in set 04 showed major intensification in SOD, CAT and GSH levels while significant decrease in the level of MDA was observed in set 03.

Table.3: Effect of melamine used alone or with Costus extract on Mean + SD on hepatic antioxidant enzymes in rats.

| Parameters | Set 01 (control) | Set 02 (Costus) | Set 03 (MA) | Set 04 (Costus + MA) |
|---|---|---|---|---|
| SOD | 6.04±0.23 | 6.09±0.23 | 3.03 ± 0.76* | 6.01±0.01** |
| CAT | 10.08±0.16 | 10.02±0.16 | 6.02 ± 0.91* | 10.09±0.09** |
| GSH | 60.32±0.44 | 60.67±0.44 | 30.07±0.06* | 60.01 ± 0.34** |
| MDA | 40.08±0.12 | 40.87±0.12 | 50.01±0.44* | 40.02±0.14** |

Number (n) per group = 20, SD = standard deviation, Set 01 (control) obtained equal volume of distilled water/day for 2 months, Set 02 will be obtained 1.5 % w/v Costus plant extract (0.4mg/ kg) by mouth for two months, Set 03 obtained MA (50mg/kg body weight /day) for 2 months, Set 04 obtained MA (50mg/kg body weight /day) plus Costus plant extract (0.4mg/ kg) by mouth for the duration of two months. Glutasthione, SOD: Super Oxide, Dismutase, CAT: CAT alase and MDA: Malondialdehyde. * = p < 0.05, ** = p < 0.01.

## 3.4. Observation under Light microscopic:

Rats Liver tissue in Set 01 and 02 exhibited normal histological structures of hepatocytes comprised large spherical nucleus with marked nucleolus. Cells were arranged into hepatic cords known as blood sinusoids were lined by Kupffer cells. (Fig. 1).

The liver tissue of rats of Set 03 after administration of MA showed congested blood vessels, increased vacuolar degeneration all over cytoplasm of hepatocytes with dilation of the blood sinusoids and increased in number of Kupffer cells. In a few areas of liver tissue showed necrotic changes in the hepatocytes as small and pycknotic cellular nucleus with condensed chromatin, and reduction of nucleolus. (Fig. 2). Liver of rats treated with Costus extract in combination with MA (Set 04) showed marked recovery and restoration to almost normal hepatic tissues (Fig. 3).

## 3.5. Transmission electro microscopic observations:

The hepatocytes of rats of Set 01 and 02 showed normal ultra-structure as normal euchromatic nucleus and nucleolus with normal cell organelles as normal Golgi bodies, numerous endoplasmic reticulum and numerous mitochondria (Fig. 4).

The hepatocytes of rats of Set 03 after administration of MA alone, the transmission electron microscopic observation of hepatocytes showed marked destruction of cell organelles as nuclear membranes, decrease sized nucleolus, destruction and aggregations of mitochondria and rough endoplasmic reticulum with increase in cytoplasmic vacuoles (Fig. 5).

The hepatocytes of rats of set 04 which were exposed to MA in combination with Costus extract showed marked recovery and restoration (Fig. 6).

# 4. Discussion

Hematopoietic system is a sensitive system to evaluate the hazardous effects of poisons in humans and animals (Liju et al. 2013). As regard hematological findings the present study revealed that data in Table 1 found that rats of set 03 treated with MA had significant decrease in RBC count, hemoglobin level, total WBC count and lymphocyte meanwhile neutrophils and monocytes were significantly elevated in comparison to with Set 01 and 02. These results were in agreement with those reported by (Salem et al. 2018) who reported that MA caused significant decrease in RBC count, hemoglobin level, total WBC count and lymphocyte but significantly elevation in neutrophils and monocytes as compared to control. The results were in agreement with results of other researchers like (Abd-Elhakim et al. 2016) who reported that MA caused significantly decrease in mean values for RBCs, Hb and MCHC of mice treated with MA as compared to those in the control mice they also added that reduction in the number of peripheral blood lymphocytes in mice treated with MA might be due to the toxic effect of MA on lymphoid organs. Our results were also in agreement with the findings of Haddad (El Rabey et al. 2013) who reported that MA caused a decrease in RBC, Hb and MCHC counts meanwhile a decrease in leukocytes and increase in neutrophils and lymphocytes.

Costus extract also induced enhance in the level of erythrocytes. It could be because of enhancing rate of erythropoiesis. It was revealed that co-administration of Costus extract with MA ameliorated the toxic effects of MA on rat hematology and blood biochemistry, our results were consistent with the findings of (El-Far et al. 2018) who reported that Costus improved the hematological and biochemical alterations.

As regard the liver function, our study revealed that as shown in table 2, the rats of set 03 treated with MA revealed significant improvement in the amount of enzymes including ALT, AST, TSB. It was also observed the reduction in the level of albumin in serum as compared with set 01 and 02. The elevation within the serum liver enzymes indicates as a necrotic lesion within the liver cells. It was also observed that the decrease in albumen level. It indicated that there was decrease in synthesis and excretory activities performed by the liver cells. The results were in agreement with biochemical and histopathological findings also parallel with the results of (Chain et al. 2010) suggested that MA increased levels of enzyme including ALT, AST, TSB. The reduction in albumin amount of serum as

compare with control group with the agreement of results reported (Tolba &Salama 2016). Also, results were in agreement with results of (Liju et al. 2013) who reported that MA toxicity caused significant increase in the levels of AST, ALT, and ALP as compared to control group. The current study suggested that the significant reduction in serum albumin, these results were parallel with the results of (El Rabey et al. 2013, Tolba &Salama 2016). It was reported that there was reduction in total serum protein and albumin in male rats. The supplementation was MA in the diet of male rats for the period of 28 days. The results were in agreement with other results of (Chen et al. 2009) who reported that reduction in albumin levels in the pet which given MA in contaminated food. Oral administration of Costus extract with MA caused reduction in the toxicity in serum including AST & ALT enzymes than controlled rats. These results were in agreement with other (El-Far et al. 2018, Nancy et al. 2019). As regard the antioxidants and lipid peroxidation Data showed in table 3, revealed that MA caused in the liver tissue of rats of Set 03. There was an increase in MDA activity. While decrease in amount of SOD, CAT activities and GSH level when compared with the Set 01 and 02. These results were in agreement with results of (Bhuyan &Zaman 2008) who reported that MA responsible for the increase in MDA. While also responsible for decrease in SOD, CAT activities and GSH level was observed as compared with the Set 01 and 02 also parallel with the results of (Adaramoye et al. 2008) who reported that high dose of melamine formaldehyde caused a highly significant elevation in the content of MDA while decreasing (CAT) activity by 77.4%. It was compared with the control animals. The oral administration of Costus plus MA caused reduction of MDA and elevate SOD, CAT and GSH level in liver tissue of rats compared to Set 01 and 02, these results were parallel with results of the (El-Far et al. 2018, Nancy et al. 2019). As regard histopathological changes, the current study suggested that hepatic tissues of rats treated with MA (Set 03) showed numerous degenerative changes of hepatocytes in the form of cells were enlarged. The cells were light and foam like in appearance. The cytoplasm was occupied with multiple spaces known as vacuole. Dilation of blood vessels also induced necrosis in some liver cell with small and psychotic nuclei and condensed chromatin. The results were in agreement with (El Rabey et al. 2013) who reported that MA caused necrotic changes, degeneration of hepatic tissues, massive infiltration of the lymphocytes and huge fatty changes. In addition to that (Adaramoye et al. 2008) confirmed that histological examination of the liver of rats treated with MA showed many degenerative changes including cytoplasmic vacuolization of the hepatocytes. Also, the results were in agreement with (Chain et al. 2010) who sugested that the liver of rats treated with melamine showed, necrosis. Our results indicated ultra-structural damages of hepatocytes treated by MA as the swelling of mitochondria, condensation of chromatin, and degeneration of organelles responsible for synthesis of protein in the cells as rough endoplasmic reticulum, disintegrated of cell membranes and increased number of lysosomes these were lead to increase intra cellular vacuole's cytoplasm of hepatocytes at the last cell ,these ultra-structures alteration in the liver after administration of melanin has been reported by (Salem et al. 2018) who reported that that MA treatment caused severe degenerative changes in histopathological and ultra-structural structures. On the contrary the pre-treatment with Costus extract had a valuable role in the MA-induce prior variations through antioxidant activities. It has also reported for its ability to scavenge free radicals.

# Conclusion

The findings in this study indicate that Costus extract shows a protective effect by decreasing the histopathological changes caused by MA in the liver of albino rats.

# Declarations

**Author's contribution:** The author has performed all the research items.

**Funding:** Self-funding.

**Acknowledgments:** The author extend their appreciation to the Deanship of Scientific Research at Taif University for logistically supporting this study.

**Data availability:** Available upon request.

**Conflict of interest:** The author declares that there is no conflict of interest.

**Ethical approval:** All the steps and procedures of this experiment were approved by the Research Ethics Committee, Taif University, Saudi Arabia. (approval no. 52-316).

**Consent to Participate:** Not applicable.

**Consent to Publish:** Consented.

# References

1. Abd-Elhakim YM, Mohamed AA-R, Mohamed WA (2016) Hemato-immunologic impact of subchronic exposure to melamine and/or formaldehyde in mice. J Immunotoxicol 13:713–722
2. Adaramoye OA, Osaimoje DO, Akinsanya AM, Nneji CM, Fafunso MA, Ademowo OG (2008) : Changes in antioxidant status and biochemical indices after acute administration of artemether, artemether-lumefantrine and halofantrine in rats. Basic & clinical pharmacology & toxicology 102, 412–418
3. AlSaadi BH, AlHarbi SH, Ibrahim SR, El-Kholy AA, El-Agamy DS, Mohamed GA (2018) Hepatoprotective activity of Costus speciosus (Koen. Ex. Retz.) Against paracetamol induced liver injury in mice. Afr J Tradit Complement Altern Med 15:35–41
4. Bancroft JD, Gamble M (2008) Theory and practice of histological techniques. Elsevier health sciences
5. Bavarva JH, Narasimhacharya A (2008) Antihyperglycemic and hypolipidemic effects of Costus speciosus in alloxan induced diabetic rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 22:620–626
6. Bergmeyer HU, Bergmeyer J, Grassl M (1983) : Methods of enzymatic analysis; volume 2: samples, reagents, assessment of results. Deerfield Beach, Florida, Verlag Chemie
7. Bhuyan B, Zaman K (2008) Evaluation of hepatoprotactive activity of rhizomes of Costus speciosus (J Konig) Smith. Pharmacologyonline 3:119–126

8. Chain, EPoCitF, EFSA Panel on Food Contact Materials E, Flavourings, Aids P (2010) Scientific opinion on melamine in food and feed. EFSA J 8:1573
9. Chen K-C, Liao C-W, Cheng F-P, Chou C-C, Chang S-C, Wu J-H, Zen J-M, Chen Y-T, Liao J-W (2009) Evaluation of subchronic toxicity of pet food contaminated with melamine and cyanuric acid in rats. Toxicol Pathol 37:959–968
10. Dobson RL, Motlagh S, Quijano M, Cambron RT, Baker TR, Pullen AM, Regg BT, Bigalow-Kern AS, Vennard T, Fix A (2008) Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. Toxicol Sci 106:251–262
11. Dorne JL, Doerge DR, Vandenbroeck M, Fink-Gremmels J, Mennes W, Knutsen HK, Vernazza F, Castle L, Edler L, Benford D (2013) Recent advances in the risk assessment of melamine and cyanuric acid in animal feed. Toxicol Appl Pharmcol 270:218–229
12. El-Far A, Shaheen H, Alsenosy A, El-Sayed Y, Al Jaouni S, Mousa S (2018) : Costus speciosus: Traditional uses, phytochemistry, and therapeutic potentials. Pharmacognosy Reviews 12
13. El Rabey HA, Al-Seeni MN, Al-Solamy SM (2013) : Bees' honey protects the liver of male rats against melamine toxicity. BioMed Research International 2013
14. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77
15. Gupta R (2010) Medicinal and Aromatic Plants: Traditional and Commercial Uses Agrotechniques Biodiversity Conservation. CBS publishers & distributors
16. Hau AK-c, Kwan TH, Li PK-t (2009) Melamine toxicity and the kidney. J Am Soc Nephrol 20:245–250
17. Ibrahim SRM, El NSA-DA, Asfour HZ, Elshali KZ, Shaaban MIA, Al-Attas AAM, Mohamed GAA (2019) Antimicrobial, antiquorum sensing and antiproliferative activities of sesquiterpenes from Costus speciosus rhizomes. Pak J Pharm Sci 32:109–116
18. Kumar A, Dutt S, Bagler G, Ahuja PS, Kumar S (2012) Engineering a thermo-stable superoxide dismutase functional at sub-zero to > 50 C, which also tolerates autoclaving. Sci Rep 2:1–8
19. Liju VB, Jeena K, Kuttan R (2013) Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (Curcuma longa L). Food Chem Toxicol 53:52–61
20. Lijuan W, Kupittayanant P, Chudapongse N, Wray S, Kupittayanant S (2011) The effects of wild ginger (Costus speciosus (Koen) Smith) rhizome extract and diosgenin on rat uterine contractions. Reproductive Sci 18:516–524
21. Nancy A, Raj JB, Manimekalai K (2019) Comparative evaluation of the hepatoprotective effect of costus pictus d don methanolic leaf extract and silymarin on paracetamol induced liver damage in albino wistar rats. Int J Anat Res 7:6722–6726
22. Ogaly HA, Khalaf A, Ibrahim MA, Galal MK, Abd-Elsalam RM (2015) Influence of green tea extract on oxidative damage and apoptosis induced by deltamethrin in rat brain. Neurotoxicol Teratol 50:23–31
23. Ogasawara H, Imaida K, Ishiwata H, Toyoda K, Kawanishi T, Uneyama C, Hayashi S, Takahashi M, Hayashi Y (1995) Urinary bladder carcinogenesis induced by melamine in F344 male rats: correlation between carcinogenicity and urolith formation. Carcinogenesis 16:2773–2777

24. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
25. Organization WH (2009) Infant and young child feeding: model chapter for textbooks for medical students and allied health professionals. World Health Organization
26. Organji SR, Abulreesh HH, Elbanna K, Osman GEH, Khider M (2015) Occurrence and characterization of toxigenic Bacillus cereus in food and infant feces. Asian Pac J Trop Biomed 5:515–520
27. Rumbeiha W, Morrison J (2011) A review of class I and class II pet food recalls involving chemical contaminants from 1996 to 2008. J Med Toxicol 7:60–66
28. Salem RR, Mohamed AM, El-Kenawy AE-M (2018) : Protective effect of green tea against the hematological, biochemical, histopathological and ultrastructural changes in rat liver induced by subchronic exposure to melamine. Toxicology 14
29. Sanna AK, Al-Elyani RA, Dalia MD (2012) : Histological and ultrastructural studies on the effect of Costus Plant and Amphotericin B on male lung rats infected by Aspergillus niger. LIFE SCIENCE JOURNAL-ACTA ZHENGZHOU UNIVERSITY OVERSEAS EDITION 9, 5321–5338
30. Schmidt M, Eisenburg J (1975) Serum bilirubin determination in newborn infants. A new micromethod for the determination of serum of plasma bilirubin in newborn infants. Fortschr Med 93:1461–1466
31. Schumann G, 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 327:69–79
32. Srivastava S, Singh P, Jha K, Mishra G, Srivastava S, Khosa R (2013) Antiinflammatory, analgesic and antipyretic activities of aerial parts of Costus speciosus Koen. Indian J Pharm Sci 75:83
33. Tolba AM, Salama AA (2016) Adverse effects of melamine formaldehyde on the liver, kidney and brain in rats. Der Pharma Chem 8:398–409
34. Wang C, Qin X, Huang B, He F, Zeng C (2010) Hemolysis of human erythrocytes induced by melamine–cyanurate complex. Biochem Biophys Res Commun 402:773–777
35. Yin RH, Wang XZ, Bai WL, Wu CD, Yin RL, Li C, Liu J, Liu BS, He JB (2013) The reproductive toxicity of melamine in the absence and presence of cyanuric acid in male mice. Res Vet Sci 94:618–627
36. Yin RH, Liu J, Li HS, Bai WL, Yin RL, Wang X, Wang WC, Liu BS, Han XH, Han J (2014) The toxic effects of melamine on spleen lymphocytes with or without cyanuric acid in mice. Res Vet Sci 97:505–513
37. Yin RH, Li XT, Wang X, Li HS, Yin RL, Liu J, Dong Q, Wang WC, Yuan J, Liu BS (2016) The effects of melamine on humoral immunity with or without cyanuric acid in mice. Res Vet Sci 105:65–73
38. Zhou W, Jiang Y, Shi H, Dai Q, Liu J, Shen C, Yang H (2010) The characteristics of immune system changes in children who ingested melamine-contaminated powdered formula in China. Int J Environ Health Res 20:289–297

# Figures

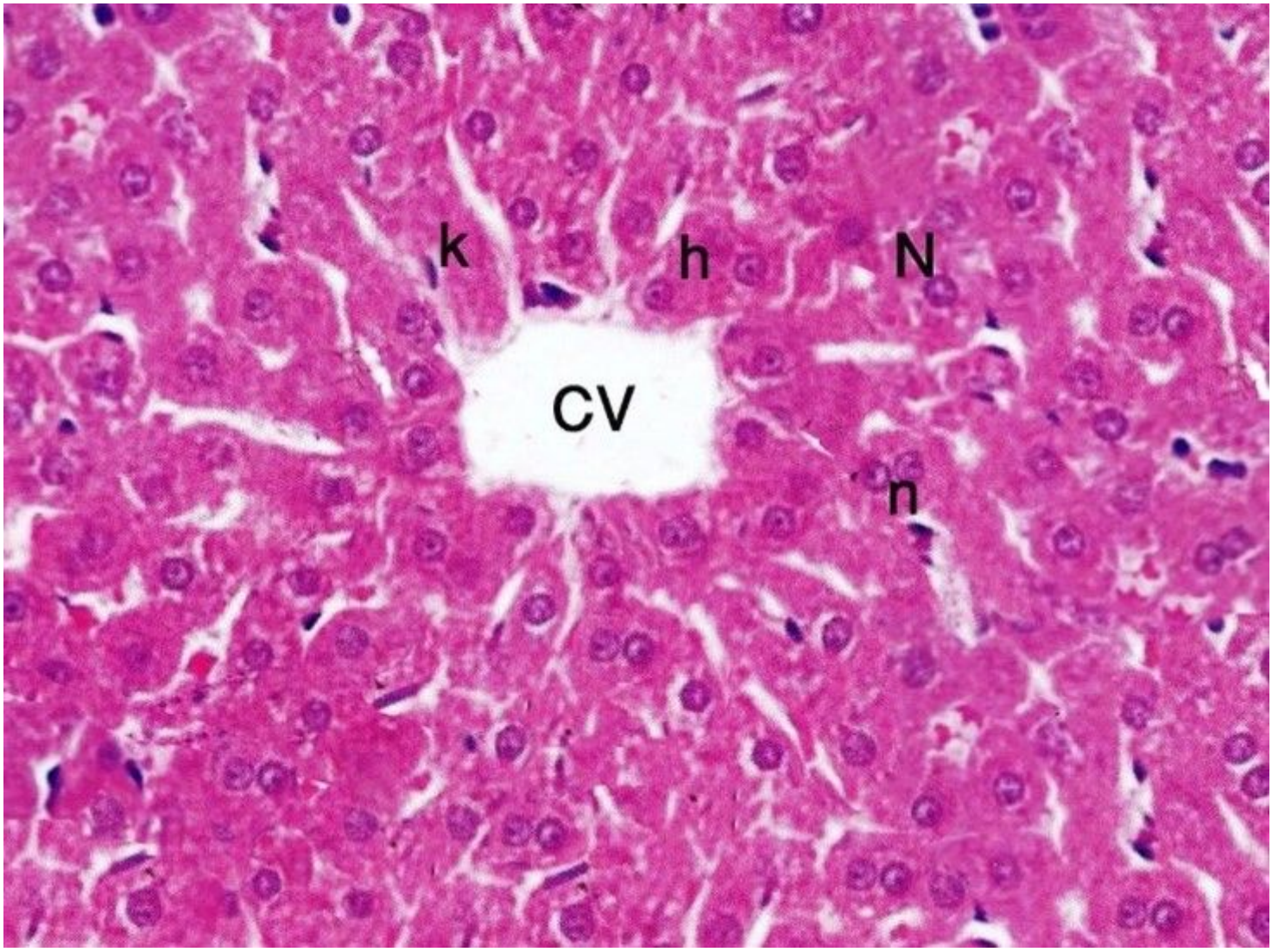



**Figure 1**

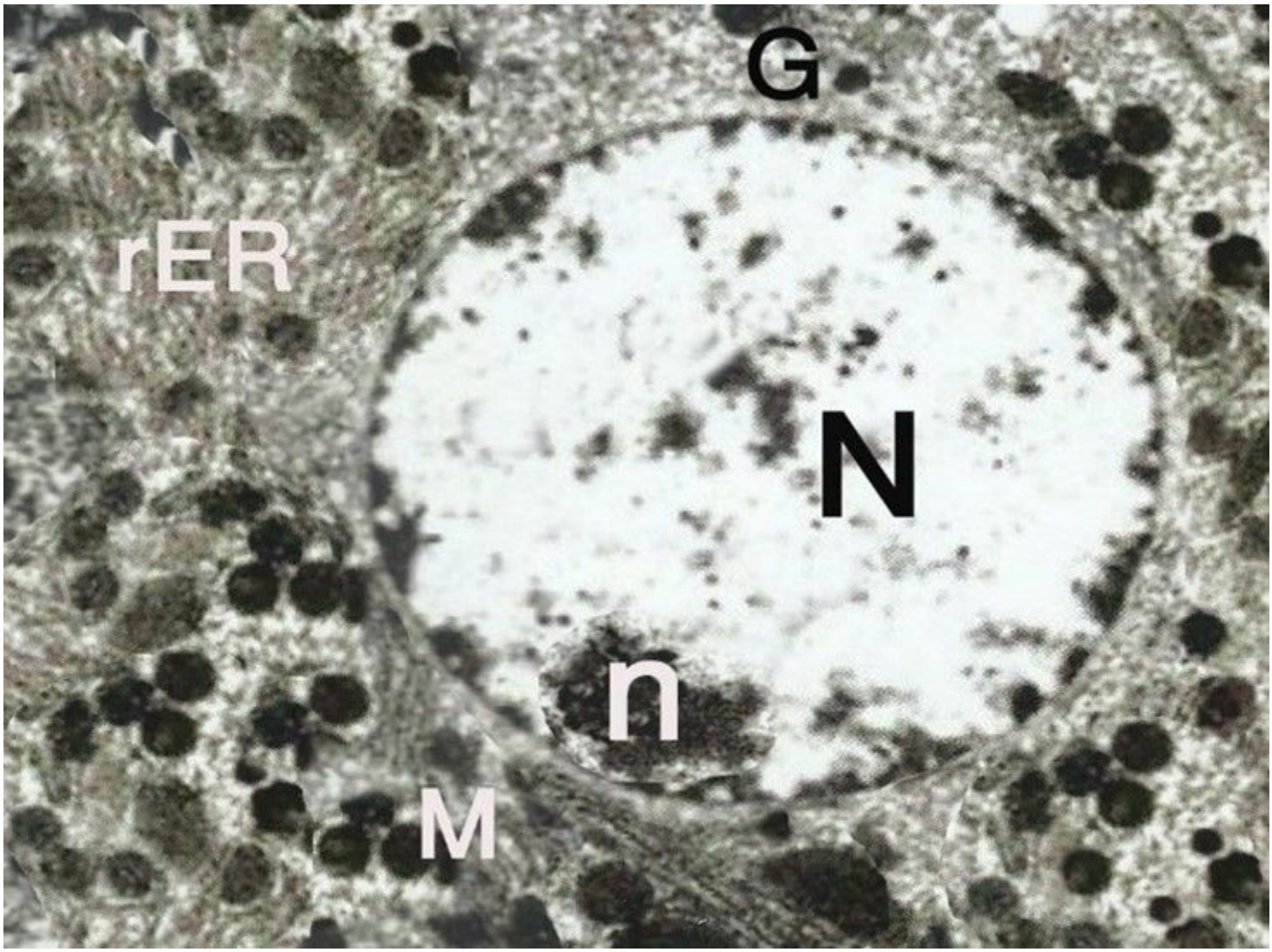
Light photomicrograph image of control rat's liver. It showed the hepatocytes (h) are hexagonal in shape with rounded nucleus (N) some cells containing double nuclei (n), cells were organized in the form of hepatic cords run in central vein (CV). Cells separated from nearby blood sinusoids (S) that contain Kupffer cells (k). The magnification was (H&E x 400).

**Figure 2**

Light photomicrograph image of rat's liver. The micrograph was taken after 8th week of exposure with MA expressed shrunken hepatocyte (h) with vacuolated cytoplasm (v), widening of blood sinusoids (S) and the central vein (CV). Notice there are degenerated areas (d) containing pyknotic nuclei (p) The magnification was (H&E x 400).

## Figure 3

Light photomicrograph of rat's liver. The photograph was taken after the 8th week of exposure with Costus extract in combination with melamine. The hepatocytes appeared nearly normal (h) arranged near central vein (CV), decrease widening of blood sinusoid (S) containing Kupffer cells (k). The magnification was (H&E x 400).




## Figure 4

Transmission electron micrograph (TEM) of control rat's liver. The image showed euchromatic nucleus (N), nucleolus (n), Golgi bodies (G), rough endoplasmic reticulum (rRE), numerous mitochondria (M). (TEM mag. = 8000X).

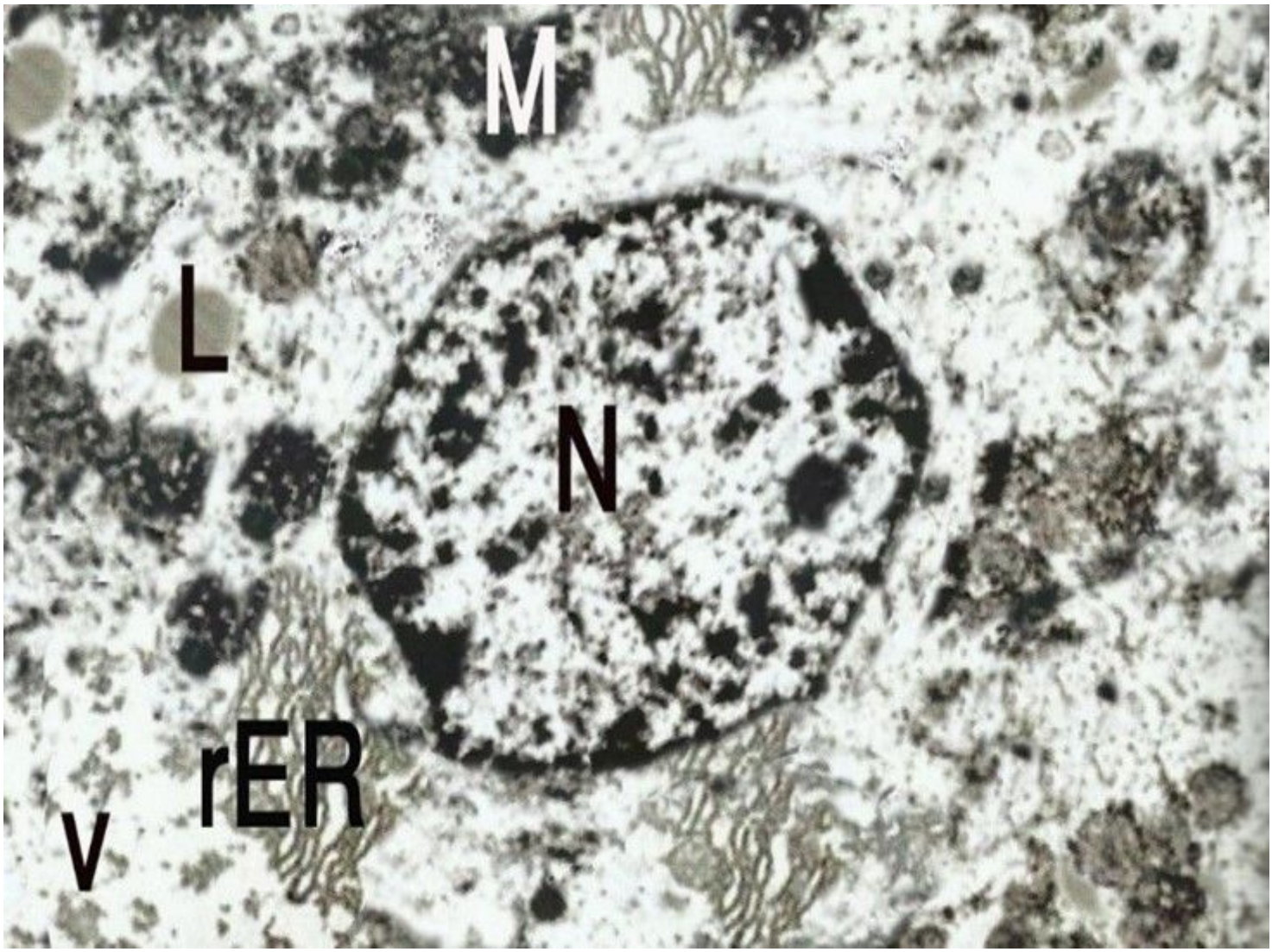



**Figure 5**

Electron micrographs hepatocytes of rats treated with melamine for 8 weeks showing destruction of cell membrane of liver cell with decreased size of its nucleus (N) and less prominent nucleolus (n), swollen and vacuolated mitochondria (M), large fat droplets (f), many cytoplasmic vacuoles (V), swollen in rough endoplasmic reticulum (rRE) and degenerated Golgi apparatus (G) and some degenerated hepatocytes (D). (TEM mag. = 10000X).

**Figure 6**

Transmission Electron micrograph (TEM) of rat's liver. It exposed with Costus extract in combination with melamine for 8 weeks. The micrograph showed N stands for euchromatic nucleus, n stands for prominent nucleolus, G stands for Golgi bodies, rRE stands for rough endoplasmic reticulum, M stands for numerous mitochondria. (TEM mag. = 8000X).