

SUPPLEMENTARY MATERIAL

Materials and Methods

Different tests for the identification of terpenoids, alkaloids, saponins, tannins, flavonoids and anthraquinone in *CMHE* were performed following reported methods [1].

*2.3. Preliminary Qualitative Phytochemical Screening***2.3.1. Glycosides Screening**

1.2g of *CMHE* was hydrolyzed by 10 ml of 1% HCl solution and neutralized with 10% of NaOH solution followed by addition of few drops of Fehling's A and B solutions. Presence of glycoside was indicated by formation of red precipitate.

2.3.2. Alkaloidal Screening

200 mg of *CMHE* was heated on a boiling water bath with 5mL of 2N HCl. Cooled mixture was then filtered and divided into two equal portions. One portion was treated with few drops of Mayer's reagent and other with Dragendorff's reagent. Presence of alkaloids was indicated by turbidity of resulting precipitates.

2.3.3. Tannins Screening

Approximately 200 mg of *CMHE* was mixed with 10 ml of distilled water and heated on water bath. Later it was filtered and 5% (w/v) solution of ferric chloride was added in the filtrate. Presence of tannins was indicated by formation of dark green solution.

2.3.4. Saponins Screening

Approximately 200 mg of *CMHE* was shaken with 5ml of distilled water in test tube and heated on water bath to boil. Presence of saponins was indicated by formation of strong and stable foam.

2.3.5. Flavonoids Screening

Approximately 200 mg of *CMHE* was dissolved in diluted NaOH and 1M of HCl (5ml each). Presence of flavonoids was indicated as yellow solution turned colourless.

2.3.5. Terpenoids Screening

200 mg of *CMHE* was mixed carefully with 2ml of CHCl_3 and 3 ml of concentrated H_2SO_4 . Presence of terpenes was indicated by formation of reddish brown coloration of the solution at

interface.



Figure S1. Standard curves of reference compounds. Where A = blank, B = benzoic acid, C = caffeic acid, D = chlorogenic acid, E = cinnamic acid, F = ferulic acid, G = gallic acid, H = coumaric acid, I = sinapic acid, J = syringic acid and K = vanillic acid.

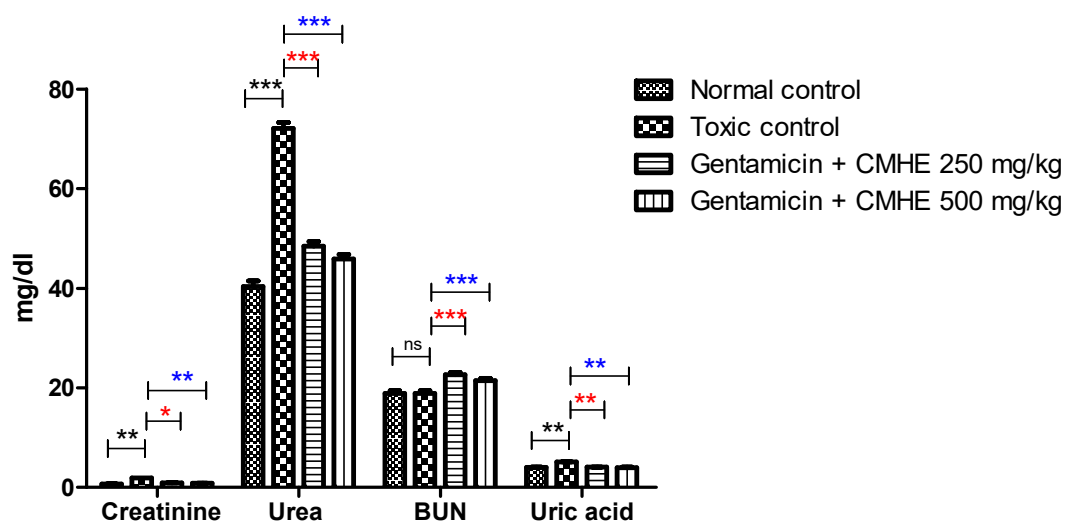


Figure 2. Graphical representation of biochemical parameters. Expressed as mean \pm S.D. $n = 6$, where, black asterisks = normal control vs. toxic control; red asterisks = toxic control vs. gentamicin + CMHE 250 mg/kg; blue asterisks = toxic control vs. gentamicin + CMHE 500 mg/kg. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

References

1. Tadesse, G. Reneela, P. Dekebo, A. Isolation and characterization of natural products from *Helinus mystachnus* (Rhamnaceae). *J. Chem. Pharm. Res.* **2012**, *4*, 1756–1762.