2- the experiment, rats were sacrificed after overnight

Design

2- Materials and Methods1. Materials: Rats and Diet: Male albino rats of Sprague Dawley strainweighing 170±10g were purchased from the Laboratory Animal Colony, Ministry ofHealth and Population, Helwan, Egypt. Basal diet constituents were obtainedfrom El- Gomhorya Company, Cairo, Egypt. Chemicals and fed ingredient: N-Nitrosodiethyamine (NDEA) liverfunction kits were purchased from Sigma- Aldrich Co.

(St. Louis, Missouri, USA). Carbon tetrachloride (CCl4) was obtained from El-Gomhorya Company, Cairo, Egypt. Ginger waspurchased from local Market. Then , the ginger dried and powdered.

2. Methods: 2-1 Preparation of basal diet: The basal diet (AIN-93M) was prepared according to Reeves etal.(1993). Diet was formulated to meet the recommended nutrients levels forrats. 2-2Induction of HepatocarcinomaInducing hepatocarcinoma wasdone according to (Sarkaret al., 1997; Dakshayaniet al., 2005; Singha et al., 2009).

One gram of NDEA was dissolved in 25 mL physiologic saline solution (0. 9% NaCl)and injected intraperitoneally (with least distress to both rat andinvestigator) to each rat in a single dose of 200 mg/kg body weight. Two weekslater, animals received subcutaneous injections of CCl4 dissolved in olive oil at a dose of3 mL/kg/rat body weight for 6 consecutive weeks to promote the hepatocarcinomain rats. 2-3 Experimental Design: Forty male albino rats werefed on the basal diet and water was provided ad libi tum.

Animals weremaintained under standard conditions of humidity (50- 60%), temperature (20-25°C)and light (12-h light: 12- h dark cycle) for one weekbefore starting the experimental foracclimatization. Rats were divided

intofour groups of ten animals each as follows: Group1: (N = 7) fed on Ain-93M and used as a negative control (Negative control). Group 2: Fed on Ain-93M, injected with NDEA and CCl4 according tothe above protocol and used asHepatocarcinogenesis control.

Group 3: Fed onAin-93M, injected with NDEA and CCl4 as above and administered daily 0. 5 % ginger powder, for 12 weeks. Group 4: Fed on Ain-93M, injected with NDEA and CCl4 as above and administered daily 0.

1 % gingerpowder, for 12 weeks.. Group 5: Fed on Ain-93M, injected with NDEA and CCl4 as above and administered daily 0. 2 % gingerpowder, for 12 weeks. In the first six weeks of theexperiment, animals of groups (2), (3), (4) and (5) were followed the aboveprotocol to induce hepatocarcinoma in rats. At the end of experiment after ~12 weeks of theexperiment, rats were sacrificed after overnight fasting. Blood samples were collectedas practical as possible in clean and dry tubes from the portal vein and leftto clot at room temperature (26-27 ºC).

Blood samples were then centrifuged at3000 rpm for 15 min, serum was carefully separated and kept at -20°C untilanalyses. 2-4 Biochemical Analyses3. Aspartate and Alanine Transferases (AST/ALT)AST and ALT were doneaccording to (Murray, 1984a) and (Murray, 1984b), respectively. The principal of AST assay was based on AST catalyses thereversible transfer of an amino group from aspartate to ?-ketoglutarate formingglutamate and oxaloacetate. The oxaloacetate produced is reduced to malate bymalate dehydrogenase (MDH) and NADH.

Similarly, ALT catalyses the reversibletransfer of an amino group from alanine to ?-ketoglutarate forming glutamateand pyruvate. The pyruvate produced is reduced to lactate by lactatedehydrogenase (LDH) and NADH. Reagent kits were used (Spinreact Co., Barcelona, Spain), and determined spectrophotometrically at 340nm by usingspectrophotometer (BT-260 Plus, Shanghai, China).

Theconcentration of the samples were calculated and the results were expressed as (U/L).