

histological examination. All slides were initially read by one pathologist and peer reviewed by the second pathologist. Statistical analysis

Values were expressed as mean±SD. Means of treated groups were compared with those of control groups using one-way analysis of variance (ANOVA) followed by Dunnett's test (9,12). Differences were considered statistically significant if p<0.05. RESULTS Acute toxicity Single oral doses of HESA-A up to 13.7 g/kg could not cause any morbidity or mortality in the animals.

Sub-acute toxicity

A daily oral administration of HESA-A at doses of 1250, 2500 and 5000 mg/kg to mice and rats during 30 days failed to cause any death and did not change the animal's Figure 1: Effect of different doses of HESA-A on body weight gain of rats. Wistar rats of both sexes in groups of 10 animals per each orally received vehicle (10 ml/kg) or different doses of HESA-A for 30 days. Data are mean of body weight gain (g).

general behavior and autonomic signs. Figure 1 shows the weight gain of control and test animals. HESA-A during the one month period of experiment could not affect the weight gain of animals. Table 1 depicts the drug effect on hematological parameters of mice. As it is observed, HESA-A even at the highest dose used, did not alter hemoglobin concentration, hematocrit and the number of red and white blood cells as well as the number of platelets. Table 2 shows the effect of HESA-A on biochemical parameters of rats. Serum concentration of glucose, urea, creatinine, albumin and globulin as well as the activity of marker enzymes (AST, ALT, ALP, LDH) in HESA-A treated groups were not significantly different from control values. Histologic examination of the main organs did not reveal morphological changes of tissues that could be clearly related to the treatment with HESA-A. Only a slight hepatic infiltration occurred at a dose of 5000 mg/kg. DISCUSSION According to the results of this study, it seems that HESA-A is a safe drug. The drug even at high doses did not affect the general behavior or autonomic signs of animals. The weight gain in HESA-A treated animals was not different from control animals. Many anticancer drugs damage the epithelial cells of the digestive tract, cause anorexia, nausea and vomiting, malabsorption and these effects cause weight reduction (5, 20). Since HESA-A did not influence the weight gain, it is unlikely to produce toxic changes above side effects on alimentary canal. Anticancer drugs such as aminoglutethimide, azathioprin, cyclophosphamide and methotredate cause hepatotoxicity, some drugs including cisplatin and cyclosporin produce nephrotoxicity (3,10,11,14,19). Transaminases (AST, ALT) and alkaline phosphatase are good indices of liver and kidney damage, respectively (13). The drug did not induce any damage to liver and kidney which could be inferred from normal activity of these enzymes. Since HESA-A did not alter the blood concentrations of urea and creatinine, this again confirms that the drug is not nephrotoxic. LDH activity of HESA-A treated groups were not significantly different from control group. LDH has 5 isoenzymes. The isoenzymes are composed of 2 different types of subunits, called M and H, that are combined randomly with each other in a tetrameric structure. The Table 1:

Hematological profile of mice fed different doses of HESA-A for 30 days. Groups Dose (mg/kg) Hb (g/dl) HCT (%) RBC Cells X 106/mm3 WBC Cells X 103/mm3 PLT Cells X 105/mm3 Control 0 11.2 ± 0.5 41.5 ± 1.2 5.8 ± 0.6 4.1 ± 0.4 9.1 ± 0.3 HESA-A 1250 11.5 ± 0.3 42.5 ± 1.8 5.7 ± 0.5 4.2 ± 0.4 9.0 ± 0.6 2500 11.3 ± 0.6 42.0 ± 0.9 6.1 ± 0.8 4.0 ± 0.3 8.7 ± 0.4 5000 11.5 ± 0.6 42.2 ± 1.3 5.9 ± 0.6 4.2 ± 0.6 9.0 ± 0.7 Each value is mean±SD of 10 mice in each group. Hb:

Hemoglobin, HCT: hematocrit, RBC: red blood cell, WBC: white blood cell, PLT: platelet Medical Journal of Islamic Academy of Sciences 14:4,145−149, 2001 Table 2: Effect of HESA-A on biochemical parameters of rats. Groups Dose (mg/kg) Glucose (mg/dl) Urea (mg/dl) Creatinine (mg/dl) Albumin (g/dl) Globulin (g/dl) AST (IU/ml) ALT (IU/ml) ALP (IU/ml) LDH (IU/ml) Control 0 83±3 38±3 0.41±0.05 4.15±0.11 1.95±0.08 89.7±8.6 26.0±2.9 39.5±4.3 1601±108 HESA-A 1250 79±4 42±5 0.39±0.06 4.20±0.18 1.99±0.11 95.2±10.2 27.4±5.6 37.2±5.8 1546±154 2500 81±4 39±5 0.46±0.07 4.08±0.21 2.02±0.06 103.3±22.5 31.0±6.1 37.1±4.9 1485±211 5000 78±5 41±3 0.40±0.04 4.22±0.13 1.89±0.14 94.2±14.1 26.3±2.8 38.6±5.7 1392±290 Values are mean±SD of 10 animals in each group. AST:

aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase. five major isoenzymes have the compositions M4, M3H, M2H2, MH3, and H4 with M subunits predominant in skeletal muscle and the liver and H subunits predominant in the heart (2, 4). Although the activity of different isoenzymes of LDH was not determined in our study, however, since the total activity of LDH was not altered by HESA-A, it seems that the drug did not damage the skeletal muscle and heart cells. Bone marrow depression, or myelosuppression is also common to the majority of antineoplastic agents and is probably the single most important dose-limiting adverse effect (5). HESA-A did not affect the number of blood cells as well as Hb concentration and hematocrit and therefore it is unlikely to produce bone marrow toxicity. In conclusion, since in sub-acute study, an oral dose of 5000 mg/kg of HESA-A administered for 30 days did not induce any biochemical, hematological and histopathological sign of toxicity, it can be defined as no-observed adverse-effect level (NOAEL) for mice and rats used under the experimental conditions. However, it should be emphasized that this NOAEL was derived from only a sub-acute study. For a more reliable safety evaluation performed on the basis of the acceptable daily intake concept data on the long-term toxicity, reproductive toxicity, genotoxicity and carcinogenicity of HESA-A are also required. REFERENCES

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