

**CHRONIC ORAL TOXICITY EVALUATION OF OLIVE LEAF FLOUR (*OLEA EUROPAEA* L)  
IN FEMALE WISTAR RATS ACCORDING TO OECD GUIDELINE 408**

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**INTRODUCTION:** Olive trees (*Olea europaea* L.) are primarily known for producing extra-virgin olive oil. Recent studies have emphasized the antioxidant potential of oleuropein, the main bioactive compound present in olive leaves. Despite this, the safety of consuming olive leaf by-products as dietary supplements has not yet been fully established. **OBJECTIVE:** This study aimed to assess the safety of olive leaf flour through a 90-day chronic toxicity evaluation, following the OECD Guideline 408.

**MATERIALS AND METHODS:** Fifty female Wistar rats were randomly allocated into five groups: a negative control (n=10), a positive control with cellulose fiber (n=10), and three treatment groups receiving olive leaf flour at doses of 500 mg/kg, 750 mg/kg, and 1000 mg/kg (n=10 each). The flour was incorporated into pellets and administered until complete intake was confirmed. Control groups received equivalent pellets containing either standard feed or cellulose. Throughout the 90-day period, animals had free access to water and standard diet. Clinical evaluations included monitoring of body weight, food, and water intake. At the end of the study, animals were euthanized for blood and organ collection. Hematological assessments included hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, and leukocyte profiles. Biochemical analyses comprised urea, creatinine, AST, ALT, ALP, glucose, and CK-NAC. Lipid profile evaluation included triglycerides, total cholesterol, HDL-C, and LDL-C. Immunological markers measured were interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ).

**RESULTS AND CONCLUSION:** No significant changes were found in weight gain, food and water consumption, or relative organ weights. Hematological parameters remained within normal ranges, though treated groups showed a slight increase in leukocytes (p=0.05). Biochemical markers—hepatic (AST, ALT, ALP), renal (urea, creatinine), cardiac (CK-NAC), glucose, and lipid profile—showed no relevant alterations. TNF- $\alpha$  levels were similar across all groups. However, IL-6 levels rose in a dose-dependent manner among treated and positive control animals (p<0.01). These findings indicate low chronic toxicity risk associated with olive leaf flour in female rats, though the immune response marked by IL-6 elevation warrants further study.

**Keywords:** olive leaf flour; chronic toxicity; oleuropein;

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