**Acute toxicity study**

For determination of LD50 of the ethanol extract (EE) and identified compound (IC), initially a pilot study was conducted on two groups (n = 10) of mice to select the dose ranges for subsequent study. On the basis of the results of pilot study, a narrow range of doses were selected to determine LD50 value. For LD50 estimation, there were five groups (n =20) of swiss albino mice were taken. The different doses (mg/kg) of EE and IC were administered orally (p.o., through oral gavages) to the animals. Two groups of vehicle-treated animals for oral route was kept as control. The animals were observed continuously for the first 4 h and then in each hour for the next 24 h and after administering of the EE and IC. The percentage of mortality at different doses after 24 hrs was calculated and the values were transformed into probit scale and LD50 for EE and IC was calculated [1].

References

1. Lorke DA. A new approach to practical acute toxicity testing. Arch Toxicol. 1983; 54(4): 275-87. https://doi.org/ 10.1007/BF01234480