Materials and Methods
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Materials

Healthy Swiss albino mice (*Mus musculus*) of both sexes weighing between 25-30 grams, reared and maintained under the supervision of Animal Welfare Committee, Department of Zoology, University of Kalyani, were used.

Experimental Design

Control and ultrasonicated series

Single dose ultrasonic sound exposure series

Unsonicated healthy mice were examined for their baseline chromosomal and nuclear damages and sperm head abnormalities (S1). Another set of unsonicated mice were fed with Arnica Montana 30 alone in doses described below and were scanned for the data at the same fixation intervals as insonicated lot. For homeopathic drug fed series 9 batches of 5 mice each were subjected to whole body ultrasonic sound waves. Out of 9, 3 batches were sacrificed at 2 hour, 3 at 24 hour, 3 at 48 hour. Among these 3 batches one batch of mice was orally administered with potentized Arnica Montana 30 [HAPCO, Calcutta (0.06 ml of liquid Arnica Montana 30 diluted with 20 ml of double distilled water to make the stock solution of the drug from which one drop 0.06 ml was fed thrice at an interval of 30 minutes to mice which was sacrificed at 24 hour and thrice a day at an interval of 8 hours to mice sacrificed at 24 hour)]. Another batch of mice was fed with dilute succussed alcohol [(Alcohol 30) Positive control 1] prepared as per homeopathic potentization procedure similarly at corresponding intervals of time. The third batch of mice were neither fed with the homeopathic drug nor with the diluted succussed alcohol (Alcohol 30), but was allowed to take only double distilled water at corresponding intervals (Positive control 2). For ultrasonic irradiation the machine used was an ultrasonic cell disrupter (LSL, SECFROID SA, Microsome model XL 2005, Switzerland) which generated sound waves of 22 to 23 KHz. Mice were immobilized during sonication in a thin wet cloth bag. The microprobe (tuned to vibrate at 20 KHz) was inserted into water...
slightly above the experimental specimens submerged in water in a suitable glass jar (containing just enough water, to submerge mice specimens leaving its head free of water) operating at an energy output percentage of 70. During sonication ice was placed around the glass jar containing water and the specimen, preventing water inside to become too hot during sonication. Mice were exposed to sonication for a total period of two minutes (twice for 1 minute each with an interval of 1 minute in between). Mice receiving this 2 minute exposure conformed the materials for the single exposure study Then they were sacrificed at 2 hours, 24 hours, 48 hours. A total number of forty five specimens of both sexes of mice (15 per each fixation interval) were used in the treatment series. As mentioned before another set of unsonicated healthy mice served as negative control.

Multiple Dose Ultrasonic Sound Exposure Series

Four sets of mice (each set comprising of 5 mice) were subjected to repeated exposures as mentioned above for 2 minutes each at an interval of 20 days so that mice sacrificed at 30, 60, 75 and 90 days after initial exposures to sonication respectively. In the repeated exposure series one batch of sonicated mice was orally administered with the stock solution of potentized homeopathic drug Arnica montana 30 in the same way but at an interval of 12 hour till sacrificed. Only one control was maintained in this series, that of the alcohol 30 fed control as there was no significant difference between the succussed alcohol 30 fed and distilled water fed control.

The observer was “blinded” by the supervisor during observation while scoring of the data on various cytogenetical parameters in the control and treated series (i.e. the slides for observation were coded without letting the observer know whether the slide was of the treated or control series). Mitotic index was not scored in the repeated exposure series.

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Chromosome aberration study

Mice at all fixation intervals were intrapentoneally injected with 0.03% colchicine solution at one and a half hour prior to sacrifice. The conventional citrate/ flame drying technique was followed for bone marrow chromosome preparation as described in the first chapter.
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**Micronuclei testing and mitotic index**

A part of the suspension of bone marrow cells in 1% sodium citrate solution was smeared on clean grease free slides. The slides were briefly fixed in methanol and subsequently stained with May Grunwald solution, followed by Giemsa staining as elaborated in Chapter I.

**Sperm head anomaly study**

Epididymis of each side of control and treated mice was dissected and taken out separately into 10 ml of 0.87% NaCl. The inner contents were taken out and thoroughly shaken to make the sperm suspend in saline solution. The suspension was filtered through a silken cloth to remove the debris and dropped on clean grease free slides. The slides were allowed to air dry and then stained in dilute Giemsa as per the routine procedure stated earlier in chapter I.