SAFETY PROFILE OF SILVERSOL®

Safety of SilverSol® as a topical and oral administration has been proven in several *in vitro, in vivo,* and human studies as summarized in **Appendix 1**.

Cytotoxicity in vitro: (Appendix 2)

Various cell lines- L929 (mouse fibroblast), Vitro (African green monkey kidney cells) and Hep2 Human epithelial cells) were exposed to SilverSol® different concentrations 10, 24, 32 PPM. No cytotoxic effect was seen in these experiments.

Animal toxicity studies: Acute oral toxicity (Appendix 3)

Mice: Safety of SilverSol® was confirmed in oral acute toxicity studies. None of these studies showed toxic effect in mice and rats.

Acute oral toxicity study was conducted in Swiss albino mice at the dose 50, 500 and 5000 mg/kg and animals were observed for 14 days. There was no mortality seen at the end of 14 days and animal did not show any sign of toxicity. None of the animals showed abnormal histological pattern.

Rats: Acute oral toxicity was also evaluated in 10 rats, wherein 5000 mg/kg dose was administered. There was no mortality or evidence of toxicity observed in any of the rats.

Animal toxicity studies: 28 days oral toxicity in rats (Appendix 3)

The study was conducted in Inbred albino Wistar rats ilver nanosolution was administered in three different doses, 0.5 ml, 1.0 ml, and 1.5 ml in the study groups and normal saline in the control group. The above doses were administered orally once a day for 28 days. No toxicity was observed. No drug-related clinical signs or deaths occurred. There were no changes in heamtological and histopathological findings.

Animal toxicity studies: Topical application (Appendix 3)

Delayed Hypersensitivity

The study was conducted in healthy Hartley albino guinea pigs. The guinea pigs were initially exposed to the test article extract and the control solution in an induction phase

lasting nine days. In this phase, the solutions were first injected intradermally (ID). For the ID injections, the solutions were given undiluted as well as emulsified with Freund's Complete Adjuvant (FCA). Seven days ± one day after the ID injections, the extract, vehicle blank and positive control solution were administered topically over the same area in which the ID injections were given. The topical exposure lasted 48 hours. Fourteen days ± one day after the topical administration, the animals were challenged. For the challenge phase, the extract and control solutions were administered topically to untreated sites and remained on the animals for 24 hours. The dosing sites were scored for skin reaction 24 ± 2 hours and 48 ± 2 hours after removal of the chambers. Based on the skin reaction scores observed, the test article was classified as to its allergic potential.

The study results confirmed the safety of SilverSol as According to the criteria for this test, the sensitization potential of the test article for the animals used in this study was classified as Grade 1 (no different than control).

Skin irritation test

Three New Zealand White rabbits were used in the test. One day prior to dosing, the hair on each animal's back was removed with clippers. The test article or an extract of a test article was applied in 0.5 g or 0.5 mL portions (or 25 mm x 25 mm x not greater than 5 mm patches, for solids) to the intact clipped skin sites of each animal. To protect the dosing sites, the trunk of each animal was wrapped with a semi-occlusive gauze bandage, which was held in place with Zonas Porous tape.

After a minimum of 4 hours of exposure, the animals were unwrapped, and the test article was removed. The test sites were observed and scored 1, 24, 48, and 72 hours after removal of the test article. Based on the 24, 48, and 72 hour scores, a Primary Irritation Index was calculated, and the response to the test article was assigned a descriptive rating. The results indicated that the irritation response category of this test article is classified as negligible.

Human studies: (Appendix 4)

Colloidal elemental SilverSol® 15 ml of 32 ppm when given orally for 14 days (480µg/day), produces no demonstrable clinically significant changes in metabolic, hematologic, urinary, physical findings.[1] The findings were confirmed in another study

by the same group and demonstrated no effect on pharmacokinetic interference on selected cytochrome P450 enzymes.[2] The authors inferred that silver metallic particle absorption is similar to that of gold nano particle, and that the silver detected in serum in human subjects is first solubilized from ingested colloidal form in the upper gastrointestinal tract and absorbed into blood in ionic form. Serum levels of ionic silver in 60 healthy volunteers were 6.8 ± 4.5 µg/L [1]. So only 1.4% gets absorbed.

In summary, the remarkable safety of SilverSol® is due to its unique tetrahedral structure that does not allow free silver to fall apart which will be taken up by the tissues and cause Argyria. Moreover, it is effective at very low concentration 32ppm. The remarkable safety of SilverSol® is due to its unique tetrahedral structure that does not allow free silver to fall apart which will be taken up by the tissues and cause Argyria. Moreover, it is effective at very low concentration 32ppm. Topical application of SilverSol® is safe. As shown in skin tests, it does not cause skin irritation or hypersensitivity. There is no risk of irreversible blackening of skin called Argyria on long term use. [1]

Safety of Toothgel: It can be inferred from human studies described above, dental application of 1 gm 22 ppm toothgel *(*22 µg silver) /day will be safe and would not cause irritation like issues. It will not have risk of the development of Argyria due to poor absorption of metallic silver. Even if silver is absorbed, serum levels would be 1.4% (of 22 µg) i.e. 0.003 µg/L (by calculation). These low levels would be clinically insignificant.

As per the Environmental Protection Agency (EPA) standards, 0.014mg//kg body weight silver per day (982 µg/day for a 70 kg adult) can safely be consumed by an adult. (Appendix 5) (https://iris.epa.gov/static/pdfs/0099_summary.pdf, https://www.meditec.se/EPA.pdf)

1. Munger MA, Radwanski P, Hadlock GC, Stoddard G, Shaaban A, Falconer J, Grainger DW, Deering-Rice CE (2014) In vivo human time-exposure study of orally dosed commercial silver NPs. Nanomed Nanotechnol Biol Med 10(1):1–9. https://doi.org/10.1016/j.nano.2013.06.010
2. Munger, M., et al., *Assessing orally bioavailable commercial silver nanoparticle product on human cytochrome P450 enzyme activity.* Nanotoxicology, 2015. **9**(4): p. 474-81.