The Safety Profile Studies of Kajal in Rabbits

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Abstract: Kajal was analyzed chemically using standard method of AOAC and found Zinc (19.81%), Sulfur (2.98%), Total Ash (46.96%) and no Lead, Cadmium, Antimony, Mercury and Arsenic contents. The safety profile studies were carried out in rabbits (both male and female) by ocular route according to the standard method. The biochemical and elemental analysis of blood samples were carried out after 0, 45 and 90 days. All the animals exhibited normal physiological activities during the whole studies. Zinc levels remained constant throughout the experimental period in all test and control rabbits. No sulfur was detected in any group, despite its minor presence in the applied sample. No mortality of animals occurred and all animals remained healthy and active throughout the experimental period.

Key words: Kajal • Safety profile studies • Elemental analysis • Blood biochemistry

INTRODUCTION

Eyes are the most expressive feature of the face and to accentuate this feature kajal is use since antiquity. The use of kajal was nearly universal and mostly applied by women across North Africa, Middle East, South East Asia, Rome and Greek Empire to enhance their charm and beauty. The black cosmetic (kajal) remnants were also found in the tombs of Egypt and China [1-3].

Traditionally kajal was usually based on Galena (Lead sulfide) and stibnite (Antimony sulfide) a black pigment. Despite of alarming effects related to kajal, it is still widely used in India, Pakistan, Middle East and also spread throughout the world by the Asian immigrants with a view of persisting cultural practice.

The aims and objectives of this research work were not only to authenticate or contradict the previous inadequate information/findings related to kajal but also to bring forward some products which are safe and can be freely used in our glamorous world.

MATERIALS AND METHODS

Chemicals and Reagents: All chemicals and reagents used in the present study were of analytical grade (Merck). Triple distilled water was used for the preparation of all solutions and standards. All glassware used was carefully cleaned first with dilute nitric acid and then rinsed with distilled water.

Elemental Analysis: The sample of Kajal was analyzed for the detection of Zinc (Zn), Sulfur (S), Lead (Pb), Cadmium (Cd), Antimony (Sb), Mercury (Hg), Arsenic (As) and Total Ash content. The elemental analysis was carried out on Atomic Absorption Spectrophotometer (Hitachi model Z-5000) equipped with Zeeman Background Corrector and a Data Processor. Estimations were made using standard addition technique. The total ash content was determined using standard method of AOAC [4].

TOXICITY STUDIES ON RABBITS

Selection of Animals: The toxicity studies were carried out in young, healthy, adult male and female rabbits weighing between 1.25-1.5 Kg according to standard method. All animals were separated on the basis of sex and kept in quarantine for 15 days. The standard diet contained lucerne, cucumber, carrot and grains. They were daily observed for their behavioral pattern and health condition. The animals showing any sign of ill effect were rejected and not included in the study.
Study Protocol: A total number of 30 animals (15 male and 15 female) were selected for the study. The animals were divided into three groups.

**Group 1:** The animals of this group received single application of the sample (about 8µg/eye) through an applicator provided with the sample in both the eyes daily for 90 days.

**Group 2:** The animals of this group received double application (16µg/eye) in both the eyes daily for 90 days.

**Group 3:** This group served as a Control group and did not receive any application.

Daily observation of the effect of test material on general health, body weight, growth, behavioral pattern, physical changes and mortalities were recorded.

Blood samples were collected from marginal ear veins of male and female rabbits from each group at 0, 45 and 90 days of sample application and were analyzed for Zinc and sulfur content on Atomic Absorption Spectrophotometer. These blood samples were also subjected to biochemical analysis.

Chronic toxicity of the sample was studied in rabbits through local application of Kajal in single and double dose as mentioned above. The test animals did not exhibit any significant effect of the sample on body weight and physiological activities, in either dose, over the period of 90 days. All test animals behaved in the same manner as that of control and showed an average increase of about 2% in their body weights (Table 2). There was no mortality and all animals remain healthy and normal throughout the experimental period.

The elemental analysis of blood samples taken from the marginal vein of rabbit’s ear at different time interval, as shown in table 2, revealed the presence of zinc only and no sulfur was detected despite its presence in the sample in a similar manner as it was observed in rats. The zinc levels, however, remained constant throughout the experimental period in all test and control animals.

Blood Analysis: Biochemical analysis of rabbit’s blood taken after 0, 45 and 90 days of experimental period has not shown any significant change in any group. All liver and kidney function tests were normal, indicating no effect of the sample on the vital organs (Table 4).

RESULT AND DISCUSSION

The study was conducted to ascertain whether the continuous use of kajal, an eye cosmetic containing zinc and sulfur, has any effect on eye locally or if there is any possibility of its absorption through eye into the systemic circulation and subsequent damage of vital organs. Topical application of the kajal in rabbits (up to 16µg/eye daily) for 90 days did not result in any redness, chemosis, discharge or swelling of the eye and thus documents its safety. Continuous use of kajal made the eyes clean, bright and shiny which may be due to the oily nature and adsorptive action of the test sample.

The kajal is applied on the upper and lower lids of the eyes and being oily in nature and due to its adsorptive property it cleans the eye surface and accumulates at the extreme edges of the eyes from where it is wiped easily [5].

Zinc appears to play an integral role in maintaining ocular function and is present in higher concentration in ocular tissues as compared with other tissues [6, 7]. The highest concentration of zinc was found in retina and choroids, which is followed by ciliary body, iris, optic nerve, sclera, cornea and lens [8-0]. In rodents zinc has been found to be localized in photoreceptor cells and its deficiency leads to cataract [11]. Active transport of zinc into portal blood is mediated by metallothionine [12]. Serum and plasma zinc concentration in adult human ranges from 80-150 mg/dl. Zinc is integral component of about 200 metalloenzymes, alkaline phosphatase, hormones and somatmedin. Toxic dose of zinc inhibit alkaline phosphatase, liver catalase and cytochrome oxidase [13]. The present study reveals that no such reaction takes place even after prolonged use of kajal in rabbits. All liver and kidney function tests were normal. This indicates that there is no toxic effect of the sample on internal organ. The sample is not absorbed into the systemic blood circulation through local application as the eye is separated from general blood circulation by two barriers, the aqueous barrier and vitreous barrier [14, 15]. This is also confirmed by the elemental analysis of blood sample. The zinc levels remained constant throughout the whole experimental period in all test and control rabbits. Present findings are in accordance with earlier studies conducted on surma [16]. It was concluded that the use of kajal as an eye cosmetic is safe.
REFERENCES