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Studies of an Eye Cosmetic in Experimental Animals

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Abstract: Animal studies have been carried out to evaluate the safety use of kajal, a universal eye cosmetic, in order to confirm or contradict the previous data, which is insufficient and confusing for its limitation to use as cosmetic and to assess its exposure, susceptibility and risk. Hashmi kajal tube (net weight 4.5g), an eye cosmetic manufactured by M/s A.Q. and company international Pakistan was used as source for study. The elemental analysis of Kajal was carried out on atomic absorption spectrophotometer. The safety profile studies were carried out in rats (both male and female) by oral route and in rabbits by ocular route according to the standard method. The biochemical and elemental analysis of blood samples was carried out at 0, 45 and 90 days. *Kajal* was analyzed chemically using standard method of AOAC and found to contain Zinc (18.91%), Total Ash (37.23%) and does not contain sulfur, lead, cadmium, antimony, mercury and arsenic. All the animals exhibited normal physiological activities and an increase in body weights of both test and control groups. Zinc levels remained constant throughout the experimental period in all test and control rabbits. There was no mortality and all animals remained healthy and active throughout the experimental period. Long term application (90 days) of kajal in single and double doses showed no effect on body weight, health and normal behavior of the experimental animals group as compared to the control group and there was no mortality during the experimental period. Furthermore, liver and kidney function tests were normal, indicating non-toxic effect of the kajal on vital organs.

Key words: Kajal · Toxicity · Elemental analysis · Safety profile

INTRODUCTION

The geographic origin, climatic conditions and the market requirements are some important factors influencing the variation in composition of different preparations of eye cosmetics. Kajal and kohl usually consists of salts of heavy metals such as galena (lead sulfide) and stibnite (antimony sulfide) a black pigment [1].

Kajal and kohl (surma) have been used traditionally in some parts of the world for a number of reasons. For example to enhance the appearance and beauty of the eyes, to protect the new born from the glance of evil eyes, to deter malicious spirits from women during labor, to provide relief from glaring sun and reflection from the sand, etc [2].

Presently many companies are preparing kohl and kajal. These commercially available formulations are usually based on methods derived from folklore including some herbs which have beneficial effects like prevention of eye sickness, fatigue and visual improvements [3] and some harmful substances including lead, toxins etc. which may cause untoward effects on healthy eyes [4-7].

The present study is designed to evaluate kajal for its general safety profile and long term safety profile to find out that what physiologically significant changes occur in blood and its effects on other vital organs after long term application in eyes.

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MATERIALS AND METHODS

Chemicals and Reagents: Hashmi kajal tube (net weight 4.5g), an eye preparation, is manufactured by M/s A.Q. and company International Pakistan under the license of M/s Muhammad Hashim Tajir Surma Pakistan. The ingredients of Hasmi kajal tube, as informed by the manufacturer, are zinc oxide, waxes, *Cinnamomum camphora*, processed carbon black and clarified butter.

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: Kajal was analyzed for the detection of zinc (Zn), sulfur (S), lead (Pb), cadmium (Cd), antimony (Sb), mercury (Hg), arsenic (As) and total inorganic matter (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 [8].

Toxicity Studies on Rats

Selection of Animals: The toxicity studies were carried out in young, healthy, adult male and female rats of Sprague Dauley strain, weighing between 130-140g. The animals were divided on the basis of sex and housed separately. All the animals were fed on standard diet containing grain, cereal, bread and milk supplemented with vitamins. They were daily observed for their behavioral pattern and health condition for a period of 15 days. The animals showing any sign of ill effect were rejected and not included in the study.

Preparation of Oral Feeding: To conduct the study test sample was prepared by dissolving Hashmi kajal tube in olive oil to give a concentration of 10 µg/0.1ml.

Study Protocol: The selected animals were divided into three groups each having 10 animals (5 male and 5 female rats) and were marked as Group I, Group II and Group III. Group I and II were test groups and Group III was treated as control. The feeding experiments were carried out as follows.

Group 1: The animals were deprived of food 18 hours prior feeding experiment. Test feed containing 4.5 g of test material in a single dose was given to all animals and were carefully observed for a period of 2 weeks. Any change in their mood and behavioral activity pattern was recorded.

Group 2: The animals of this group received 0.1 ml (10 µg) solution of test material daily for 90 days through oral feeding.

Group 3: The animals of this group served as a control group and fed with 0.1ml vehicle in the same manner as the test group.

Elemental and Biochemical Analysis of Blood: Blood samples were collected from 3 male and 3 female rats from each group at 0, 45 and 90 days of sample application and were analyzed for zinc content on atomic absorption spectrophotometer. These blood samples were also subjected to biochemical analysis (liver and kidney function tests).

Toxicity Studies on Rabbits

Selection of Animals: Young, healthy, adult male and female rabbits weighing between 1.25-1.5 Kg 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 designated as Group I, Group II and Group III each having 10 animals (5 male and 5 female).

Group 1: The animals of this group received single application of the sample (about 7-8 µg/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 (14-16 µg/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 eyes, general health, body weight, growth, behavioral pattern, physical changes and mortalities were recorded.

Elemental and Biochemical Analysis of Blood: Blood samples were collected from marginal ear vein of 3 male and 3 female rabbits from each group at 0, 45 and 90 days of sample application and were analyzed for zinc content on Atomic Absorption Spectrophotometer. These blood samples were also subjected to biochemical analysis (liver and kidney function tests).

RESULTS

Analysis of Sample: Elemental analysis of the sample reveals the presence of zinc 18.91% (3.02µg) only, while sulfur, lead, cadmium, antimony, mercury and arsenic were not detected and total ash content 37.23%.

Acute Toxicity in Rats: Oral feeding of sample mixed with diet in rats showed no untoward signs and symptoms during 2 weeks of observation period. All the animals were healthy, active and normal. The increase in body weights was, however, depressed as compared to the control group.

Chronic Toxicity in Rats: The chronic toxicity studies revealed no gross pathological changes in vital organs. A gain in body weights was observed in all groups over the period of 90 days. Elemental analysis of blood samples after 0, 45 and 90 days revealed no significant change in zinc levels of test and control groups. Biochemical studies of blood of rats (Table 1) shows no change in SGPT level of group 2 as compared to the control group.

DISCUSSION

The present study was conducted to assess the local application/use of kajal tube, eye cosmetic containing zinc and to find out the effects of kajal on systemic circulation after absorption through eye and to ensure subsequent effect on vital organs.

Long term (90 days) application of Hashmi kajal tube in concentration upto16 µg daily did not produce any untoward effects like redness, swelling, discharge, irritation, chemosis etc. and thus the safety of the product in small dose is confirmed. In contrast the prolonged use of Hashmi kajal tube imparted beneficial effects on eyes. The eyes of the test group were found to be clean, bright and shiny as compared to the control group. These beneficial effects can be attributed to the ingredients and the oily nature of the product. The non-hydrophilic nature produces the adsorptive action which leads to astringent effect and also prevents the absorption through cornea or conjunctiva. The adsorptive nature not only cleans the eyes but it accumulates dust or dirt which enters the eyes from extreme edges [9].

Table 1: Elemental Analysis of Sample Kajal

Sr. #	Name of Element	Symbol	Elements detected
01.	Zinc oxide %	Zn	9.56 %
02.	Sulphur %	S	Not detected
03.	Lead %	Pb	0.09%
04.	Cadmium (ppm)	Cd	0.72%
05.	Antimony (ppm)	Sb	Not detected
06.	Mercury (ppb)	Hg	Not detected
07.	Arsenic (ppb)	-	Not detected
08.	Total Ash %	-	41.01 %

Table 2:Analysis of blood drawn from rats at base line (0 days)

		Group 2		Group 3	
S. No.	Biochemical Test	Female	Male	Female	Male
01	Body weight (g)	140.0±1.80	130.8±2.40	130.0±1.80	140.0±2.00
02	Zn (µg/dl)	245±1.5275	250±1.00	246±3.785939	242±2.645751
03	Total Bilirubin (mg/dl)	0.80±0.0264	0.99±0.0152	0.76±0.0435	0.86±0.0208
04	SGPT (U/L)	43.0±3.6055	42.0±2.6457	42.0±5.2915	46.0±3.6055
05	SGOT (U/L)	132.0±3.6055	114.3±0.5773	133.0±5.2915	138.0±4.5825
06	Alkaline phosphatase (U/L)	452.0±5.2915	312.3±4.5092	250.3±2.5166	316.0±4.5825
07	Urea (mg/dl)	431.3±2.0816	313.0±2.0000	367.6±2.0816	291.0±0.0000
08	Creatinine (mg/dl)	1.13±0.2306	1.016±0.1527	0.80±0.06245	0.90±0.0435
09	Total protein (g/dl)	5.2±0.2645	5.4±0.5567	5.8±0.2645	5.2±0.36055
10	Albumin (g/dl)	2.8±2.0000	2.9±0.5567	2.9±0.4582	2.6±0.10000

Table 3: Analysis of blood drawn from rats after 45 days

		Group 1		Group 2	
S. No.	Biochemical Test	Female	Male	Female	Male
01	Body weight (g)	147.0±2.40	135.0±2.40	135.4±1.60	146.4±2.20
02	Zn (µg/dl)	250±3.2145	260±0.5773	252±1.5275	244±3.6055
03	Total Bilirubin (mg/dl)	0.69±0.0115	0.90±0.0264	0.70±0.0360	1.05±0.0057
04	SGPT (U/L)	51.0±2.6457	51.0±0.5773	63.0±0.0000	38.0±0.5773
05	SGOT (U/L)	141.0±1.7320	130.3±3.7859	146.0±2.6457	136.0±5.1961
06	Alkaline phosphatase (U/L)	513.3±3.5118	298.3±1.1547	368.3±2.0816	304.0±4.5825
07	Urea (mg/dl)	328.0±3.4641	392.0±8.7177	262.0±3.4641	216.0±3.6055
08	Creatinine (mg/dl)	0.90±0.1732	0.70±0.0624	0.60±0.0173	0.703±0.0642
09	Total protein (g/dl)	4.6±0.5291	4.86±0.05773	5.5±0.20000	4.8±0.2645
10	Albumin (g/dl)	2.93±0.05773	3.3±0.2645	3.03±0.11547	2.8±0.0000

Table 4: Analysis of blood drawn from rats after 90 days

		Group 1		Group 2	
S. No.	Biochemical Test	Female	Male	Female	Male
01	Body weight (g)	148.0±3.00	140.0±2.80	138.4±2.20	152.4±2.80
02	Zn (µg/dl)	250±3.0550	260±1.5275	252±3.000	245±1.1547
03	Total Bilirubin (mg/dl)	0.81±0.0321	0.83±0.0264	1.03±0.0057	0.72±0.0265
04	SGPT (U/L)	39.0±0.5773	63.0±1.000	51.0±1.5275	40.0±2.5166
05	SGOT (U/L)	162.3±1.5275	169.6±1.5275	109.0 ±3.0000	132.0±3.0000
06	Alkaline phosphatase (U/L)	372.0±4.3589	384.0±2.6457	382.0±3.4641	228.0±3.6055
07	Urea (mg/dl)	261.0±3.6055	408.0±10.4403	286.0±5.2915	292.0±5.2915
08	Creatinine (mg/dl)	0.80±0.0556	0.803±0.0680	1.00±0.10000	0.56±0.0642
09	Total protein (g/dl)	6.0±1.2124	5.1±0.4582	4.96±0.0 5773	5.0±0.36055
10	Albumin (g/dl)	3.0±0.36055	2.8±0.5567	2.6±0.26457	2.8±0.4582

Table 5: Analysis of blood drawn from rabbits at base line (0 days)

		Group 1		Group 2		Group 3	
S. No.	Biochemical Test	Female	Male	Female	Male	Female	Male
01	Body weight (g)	1552.4±2.60	1508.6±3.20	1584.2±2.80	1338.4±2.40	1465.6±2.60	1506.0±4.00
02	Zn (µg/dl)	250±4.3588	245±0.000	215±2.6457	245±3.2145	247±2.000	248±1.1547
03	Total Bilirubin (mg/dl)	0.50±0.0264	0.60±0.1135	0.65±0.0360	0.60±0.0529	0.72±0.0346	0.67±0.0360
04	SGPT (U/L)	37.00±1.0000	43.33±2.5166	41.33±2.3094	45.00±5.1961	48.00±3.6055	43.33±1.5275
05	SGOT (U/L)	50.00±2.6457	55.33±1.5275	31.66±1.5275	34.00±5.1961	62.00±4.3588	68.00±3.6055
06	Alkaline phosphatase (U/L)	70.00±3.6055	28.66±5.1316	70.00±5.5677	91.00±1.7320	91.33±2.3094	98.00±2.6457
07	Urea (mg/dl)	50.00±3.0556	31.00±1.7320	69.00±6.0827	65.00±1.0000	40.66±2.0816	36.00±1.7320
08	Creatinine (mg/dl)	1.46±0.2466	0.93±0.1607	0.90±0.0866	1.20±0.0500	1.10±0.0866	0.90±0.0500
09	Total protein (g/dl)	9.40±0.2645	8.50±0.6082	5.60±0.2000	5.40±0.1732	8.00±0.6245	6.90±0.9539
10	Albumin (g/dl)	4.50±0.3000	5.60±0.3464	4.20±0.1000	4.90±0.2645	3.90±0.8888	4.90±0.6082

Table 6: Analysis of blood drawn from rabbits after 45 days

		Group 1		Group 2		Group 3	
S. No.	Biochemical Test	Female	Male	Female	Male	Female	Male
01	Body weight (g)	1563.0±3.00	1520.2±3.60	1598.2±3.60	1400.2±2.20	1474.2±2.80	1522.0±3.60
02	Zn (µg/dl)	250±0.57735	250±1.5275	210±0.57735	250±2.0000	250±6.42910	250±0.0000
03	Total Bilirubin (mg/dl)	0.50±0.0435	065±0.0458	0.60±0.0057	0.66±0.0472	0.72±0.0360	0.70±0.0435
04	SGPT (U/L)	39.33±2.5166	44.66±3.0550	45.00±5.2915	46.00±2.6457	46.33±0.5773	45.00±5.5677
05	SGOT (U/L)	48.00±3.6055	54.00±2.6457	33.00±0.0000	36.33±1.5275	60.00±5.5677	63.66±2.0816
06	Alkaline phosphatase (U/L)	75.00±2.6457	27.33±2.0816	73.33±6.0277	92.00±2.6457	96.00±4.3588	102.0±1.0000
07	Urea (mg/dl)	50.00±7.5498	32.33±0.5773	70.00±4.3588	64.00±5.2915	41.66±1.5275	38.00±4.3588
08	Creatinine (mg/dl)	1.45±0.1322	0.83±0.0288	1.10±0.05	1.00±0.1732	0.92±0.0288	1.02±0.1040
09	Total protein (g/dl)	9.03±0.5686	8.50±0.5291	6.00±0.7810	5.70±0.1732	7.56±0.5686	6.8±0.1000
10	Albumin (g/dl)	4.53±0.1154	5.70±0.5291	4.50±0.2645	4.60±0.3605	4.20±0.3464	5.10±0.7000

Table 7: Analysis of blood drawn from rabbits after 90 days

		Group 1		Group 2		Group 3	
S. No.	Biochemical Test	Female	Male	Female	Male	Female	Male
01	Body weight (g)	1572.0±2.80	1530.8±4.10	1610.2±3.40	1422.0±3.00	1488.0±2.00	1532.2±3.20
02	Zn level (µg/dl)	253±2.51661	250±4.7258	210±0.5773	245±1.5275	252±1.15470	251±0.0000
03	Total Bilirubin (mg/dl)	0.52±0.0519	0.65±0.0624	0.62±0.0624	0.65±0.0529	0.75±0.0953	0.69±0.0321
04	SGPT (U/L)	40.00±0.0000	35.79±5.1961	45.33±1.5275	48.33±2.8867	50.66±1.5275	46.00±4.3588
05	SGOT (U/L)	50.00±2.6457	59.33±3.0550	34.33±0.5773	38.00±2.6457	59.00±3.0000	66.33±2.5166
06	Alkaline phosphatase (U/L)	73.00±3.6055	29.00±4.3588	67.00±2.0000	92.00±5.2915	95.00±3.4641	100.0±2.6457
07	Urea (mg/dl)	52.00±4.5825	33.00±2.6457	67.00±5.2915	66.00±3.6055	40.00±2.6457	39.33±0.5773
08	Creatinine (mg/dl)	1.48±0.0763	0.90±0.0500	1.00±0.1732	1.02±0.1527	1.05±0.0000	0.91±0.0360
09	Total protein (g/dl)	9.16±0.2886	8.70±0.3605	5.60±0.4582	5.53±0.0577	7.70±0.5567	7.00±0.3605
10	Albumin (g/dl)	4.40±0.2645	5.80±0.4358	4.30±0.2645	4.80±0.20000	4.20±0.0000	5.33±0.2081

Zinc, the only trace element found in Hashmi kajal tube, plays an integral role in maintaining ocular function. It is used in eye lotions and drops for the treatment of conjunctivitis. Toxic dose of zinc inhibits alkaline phosphatase, however, no such reaction was observed in our studies (Table 2-7). The herb *Cinnamomum camphora* used in the formulation possesses antiseptic action and is used externally for soothing and pain relieving.

Long term application (90 days) of Hashmi kajal tube in single and double application has no effect on body weight, health and normal behavior of rabbits of test groups as compared to the control group. There was no mortality during the experimental period. Furthermore, liver and kidney function tests were normal, indicating non-toxic effect of the sample on vital organs. The zinc levels of test and control group animals remained normal throughout the experimental period.

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