

Protective effect of *Kohl-Chikni Dawa* against galactose-induced cataract in rats

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SUMMARY

The efficacy of *Kohl-Chikni Dawa (KCD)*, a compound ophthalmic formulation of Unani medicine, to control cataract development was explored in rats fed 25% galactose with the diet for 6 months. When one drop of 3% *KCD* solution was applied (once and twice daily) in both eyes for 6 months in galactose-fed rats, a significant reduction of lens opacification was noticed. The morphological changes of the lenses were observed every month by slit-lamp biomicroscope. These results suggest that the local application of 3% *KCD* solution possesses anticataract effect in galactose-fed rats.

Key words: *Kohl-Chikni Dawa*; Galactose-fed rats; Anticataract activity

INTRODUCTION

Cataract is an incurable and irreversible phenomenon of lens opacification of the eye. In spite of various surgical advances in modern medical science there is lack of drugs having preventive or curative effects on this disease. The efficacy of many traditional herbal medicines to cure eye diseases is now being gradually recognized in modern medical science as well (Biswas *et al.*, 2001). How far the Unani herbal drugs could really be helpful in such cases with minimum adverse effect is of interest for thorough studies.

Kohl-Chikni Dawa is a compound herbo-mineral ophthalmic formulation (*Kohl, Surma*) used in Unani medicine invented by renowned Unani physician Shareef Khan (Khan, 1903). Various Unani physicians have long been used this herbo-mineral formulation for the treatment of premature senile cataract and corneal opacity (Khan, 1870, 1873). The drug is said to possess the property of "cauterization" and no adverse effect or reaction of the drug is reported in Unani literature (Anon, 1981). We have recently studied/demonstrated

that local application of *KCD* has potential to delay the naphthalene (Siddiqui *et al.*, 2002) and alloxan-induced cataract (Siddiqui *et al.*, 2003) in rats and found non-toxic in rabbits eyes (Siddiqui *et al.*, 2001).

The present study was undertaken to investigate the morphological bases for the effects of *KCD* on the lens in galactose-induced cataract in rats. The aim and objective of this study is to find effective dose of *KCD* and its ameliorating potential in cataractogenesis as a supporting evidence of its anticataract property.

MATERIALS AND METHODS

Drugs

The ratio (1:20:1) of the constituents used in *KCD* includes copper sulphate (S.D. Fine Chem. Ltd. Mumbai), Hard soap (M. S. factory, Aligarh) and resin of *Shorea robusta* (collected from market and authenticated at Department of Chemistry, Faculty of Science, Hamdard University, New Delhi). Galactose (S. D. Fine Chem. Ltd, New Delhi) and normal saline (0.9% NaCl) was purchased from Core Health Care Ltd, Gujarat. Tropicacyl plus (Tropicamide 0.8%, Phenylephrine 5%) (Sunways Pharmaceuticals Ltd) was used for the dilatation of pupil.

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Preparation of Kohl-Chikni Dawa

KCD was prepared after proper identification and quantification of the constituents under aseptic conditions as per the method described (Kabeeruddin, 1938). Hard soap was cut into small pieces and heated in an iron pot. As the soap started to melt, copper sulphate was added and allowed a complete liquefaction. Resin powder of *Shorea robusta* was then added and heated till the drug got burnt and converted into dry ash. After cooling, the drug was powdered by using a grinder and filtered through a sieve (size 120 m). The filtrate (micro-fine powder) was collected in a bottle and labeled as *Kohl-Chikni Dawa*. Fresh solution (3%) (1 ml contain 45 mg) of KCD in distilled water was prepared before instillation.

Animals

Fifty albino rats (female, 100-130 g) bred in the Central Animal House Facility of Hamdard University were used for the study. The animals were divided in-group of 10 each and housed under standard laboratory conditions with food and water provided *ad libitum*. The animal ethics committee of Hamdard University approved the study protocol.

Treatment schedule

The experiment included five groups (oral/local): (I) Normal saline/normal saline (twice daily); (II) Normal saline/KCD (twice daily); (III) 25% galactose food/normal saline (twice daily); (IV) 25% galactose food/KCD (once daily) and (V) 25% galactose food/KCD (twice daily). One drop of 3% KCD solution (once or twice daily) was applied locally in both eyes of the animals for 6 month between 9:00 and 17:00 h.

Induction of cataract

Cataract induction was achieved by 25% galactose food with free access of water for a period of 6 months. This galactose diet was prepared by mixing the standard laboratory food with galactose.

Determination of cataract opacification

Morphological changes in the lens were monitored by slit lamp biomicroscope (Major Surgical Co.) after dilating the pupils by Tropicacyl plus. All the

rats were lightly anesthetized with ether, and the eyes were examined every month for the development of opacities during the study for 6 months.

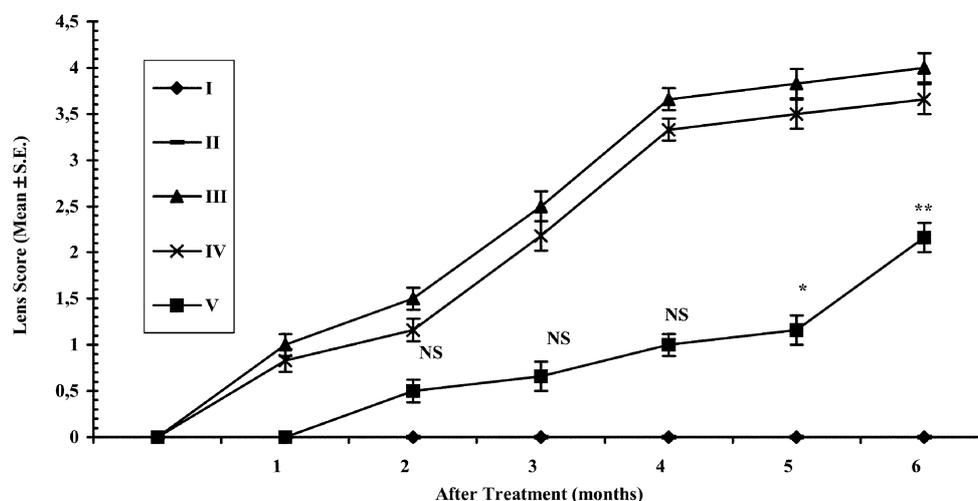
Using a modification of the classification of Sippel, 1966, the detectable changes were arbitrarily graded into four stages, as described earlier (Simard-Duquesne and Dvornik, 1973) i.e., Stage 0: lenses similar to those of rats fed chow; Stage I: faint peripheral opacity; Stage II: irregular peripheral opacity and slight involvement of the lens center; Stage III: irregular opacity involving the entire lens; Stage IV: pronounced opacity, readily visible macroscopically as a white spot. The number of lenses, which attained a given stage, was recorded. The classification of the lenticular changes was carried out by persons who were not completely unaware of the treatment status of the animals, since they had to use the nongalactosemic and the galactosemic non-treated control groups as reference. The chosen stages of lenticular changes are, however, readily distinguishable, thus minimizing the possible effect of bias on the conclusions. At the end of study, the presence of opacification was confirmed in all lenses after excising and examining them under a dissecting microscope.

Statistical analysis

The slit-images of 6 eyes in each group were used for statistical analysis. The data were expressed as mean \pm S.E.M. Non-paired student's t-test was used to analyze significance of the two means. Probability level of less than 5% was considered as statistically significant.

RESULTS

All galactose-fed rats developed cataract with similar morphological characteristics although the rate of development varied with different groups. Under slit-lamp examination, irregular peripheral opacities in the center of lens could be seen after one month in group III and by the six month these features merged to form a pronounced opacities of the lens. These opacities were readily visible macroscopically as a white spot. The opacities appeared dull whitish on examining the lenses after their removal from eyeballs.



* $P < 0.05$, ** $P < 0.01$ (statistically significant); NS (statistically non-significant) vs. Galactose fed animals.

Fig. 1. Effect of *Kohl-Chikni Dawa* eye drops on Galactose-induced cataract in rats.

The animals of group IV treated with *KCD* (once daily) developed faint peripheral opacities after one month and produced irregular and occasional pronounced opacities of the entire lens after six months. These opacities gave very dull whitish appearance on examining the lenses after their removal from eyeballs and did not found statistically significant ($P > 0.05$).

The animals of group V treated with *KCD* (twice daily) developed faint peripheral opacities in the center of lens at the end of second month and did not produced any pronounced opacities of the lens after six months. After removal from the eyeballs these opacities gave a ground-glass appearance. Comparing the morphological development of cataracts in the groups III, IV and V rats during six months of galactose feedings, the rate of cataract development was faster in group III than in group IV and V. The group V rats developed cataract at a slower pace than those of other groups, the difference being statistically significant ($P < 0.05$; $P < 0.01$). Group I and II rats treated with normal saline and *KCD* only remained clear during the six month treatment (Fig. 1).

DISCUSSION

The process of sugar cataract formation is initiated by the osmotic effects of intracellular accumulation of polyols formed by the action of aldose reductase

(AR) (Kinoshita, 1965). The concept has been substantiated by the use of AR inhibitors to control the development of cataracts in galactosemic rats (Peterson *et al.*, 1979; Beyer-Means *et al.*, 1982; Ono *et al.*, 1982). The presence of sorbitol in the lens of the human diabetic (Pirie and Van Heyningen, 1964), whether cataractous or not, suggests that the sequence of events postulated by the osmotic hypothesis may also occur in man (Chylack *et al.*, 1979). Adding to the interest in AR inhibitors is the probability that the consequences of increased AR activity in hyperglycemia may also play a role in the development of some other complications of chronic diabetes.

The galactose cataract is the easiest model of sugar cataracts to produce in rats and has been widely used as an experimental model since the initial observation of Mitchell, 1935. The rate of cataract development in rats fed galactose depends on the amount of galactose absorbed and taken up by the lens and on the rates of AR-catalyzed formation of galactitol and its elimination in the lens (Sinard-Duquesne *et al.*, 1985).

The study exhibits that galactose treated animals receiving the *KCD* solution exhibited inhibition of lens opacification in comparison to the galactose treated control group. The mechanism of this inhibition remains unclear. Standardization of *KCD*, containing 1.208% copper as one of the components, has been discussed earlier (Anon,

1986). We presume that cataract inhibition by *KCD* could be mainly due to the presence of copper in it; as Balaji *et al.*, 1992 suggested that copper is necessary for normal physiological function and is required for the activity of numerous enzymes such as cytochrome oxidase, super-oxide dismutase and uricase. The excess of copper in cataractous lenses may oxidize sulfhydryl group to disulfides followed by a reduction of copper that may generate oxygen radicals.

The role of copper in cataractogenesis has been reported variously. In one study, the copper level in the cataractous lenses in vertebrates demonstrated no correlation between copper level and cataract, and found no difference in copper levels between normal and cataractous lenses (Cook and Mc Gahan, 1986). Low level (Swanson and Trusdale, 1971) as well as elevated level (Racz and Erdohelyi, 1988) of copper has been observed in the cataractous lenses. The elevated level of copper in the lens has been elucidated to inhibit the activity of lactate dehydrogenase, which is thought to be a sensitive marker of cataract formation (Prashar and Nath, 1991). Thus, whatever be the role of copper in cataractogenesis, it is suggested that application of *KCD* eye drops twice a day may prevent the progress of cataract in rats.

CONCLUSION

The present study has demonstrated that local application of 3% *KCD* solution given anticataract effects in galactose-fed rats. Thus, the reported claim of this drug may be validated by this study. The drug is being used by people implies a relative lack of toxicity. However, drug penetration tests and controlled clinical trials will be required to confirm its anticataract activity and general safety.

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