CATARACTOUS EFFECTS OF MATERNAL USE OF HAIR DYES DURING PREGNANCY

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ABSTRACT
The purpose of the study was to investigate and compare the histopathological effects of hair dye additives 2-Amino-5-Nitrophenol (2A5NP) and 2-Nitro-p-Phenylendiamin (2NPPD) on crystalline lenses of neonates from pregnant rats that have been administered these additives subcutaneously. The study included 90 neonates of 26 nulligravida Wistar-albino rats among which ten were given 100 mg/kg/day 2A5NP (Group I), ten rats received 150 mg/kg/day 2NPPD (Group II) and rats received saline (Group III) injections subcutaneously between 7th and 15th gestational days. No sign of toxicity was observed during the treatment and there was no gross abnormality in both the study groups and the control group. Cataractous effects were seen in 26 of 30 newborn rats in Group I (86.7%), in 22 of 30 rats in Group II (73.3%) and only nine of 30 rats in the control saline injected Group III. The increased cataractogenic effect of the two additives were statistically significant when compared to the control group (Chi2:31.535, p<0.05), but there was no difference between the effects of 2A5NP and 2NPPD additives (Chi2:1.834, p>0.05). The present experimental study which was conducted in rats confirmed the cataractogenic effect of 2A5NP and 2NPPD beyond doubt. In the light of which, we can speculate that maternal exposure of hair dyes during pregnancy is potentially a risk factor to congenital cataract.

Introduction
Nowadays the hair dyes are being used increasingly for cosmetic purposes. Therefore, the potential for teratogenicity has to be established for each ingredient of the dye.

Hair dyes are separated into four groups; oxidative hair dyes, direct dyes, metal salts and natural dyes. Oxidative dyes (80%) are the most common dyes used in the US and EU. Oxidation of primary intermediates (para-phenylenediamine, para-toluenamine, substituted para-diamines, orto or para-aminophenols) and coupling with modifiers like Amino-5-Nitrophenol (2A5NP) and 2-Nitro-p-Phenyldiamin (2NPPD) result in colored reaction products (1).

Periocular dermatitis, conjunctivitis, corneal ulcers, cyclitis, secondary glaucoma, gangrene of lids, optic neuritis and proptosis are known toxic effects of hair dyes which occur upon contact to eyebrow and eyelashes or ingestion. The genetic and ocular teratogenic effects of hair dyes are not known exactly (2-4).

We planned this experimental study to investigate and compare the histopathological effects of hair dye additives 2A5NP and 2NPPD on crystalline lenses of neonates of pregnant rats.

Materials and Methods
This study was performed between November 2004 and July 2005 at Department
of Physiology, Medical School of Gaziantep University, Gaziantep / Turkey. The experiments conformed to the Principles and Guidelines for the Use of Animals in Research, Testing, and Education issued by the Committee on Educational Programmes in Laboratory Animal Science.

The study has been done on 26 female young, adult, nulligravid Wistar-albino rats. The rats were kept in six metal cages containing 3 or 5 rats each, with 20-22°C room temperatures and 12 hourly light period. In each of cages there was one male rat. After the determination of copulation by the presence of vaginal sperm each group consisting of five rats put in different cages on day zero of gestation. In 7.-15. days of gestation 10 rats were chosen randomly and 2NPPD was administrated 150 mg/kg/day subcutaneously. 2A5NP was administrated 100mg/kg/day subcutaneously to another group of 10 randomly pregnant rats. Sterile saline was injected to the control group at the same time period.

The study included 90 neonates of rats which were given 2A5NP (Group I), 2NPPD (Group II) and saline (Group III). The rats were sacrificed with intra-abdominal injection of pentothal on the 20th day of gestation. The delivery having been done with caesarian-sectio. The fetuses were inspected for any gross abnormality after drying. Thirty fetuses from each three groups of rats were sent to pathology laboratory in 10% formaldehyde buffer. The groups were coded randomly. All litters were decapitated on postnatal day 1 in the pathology department after which the eyes were enucleated and examined for histopathological characteristics with the pathologist blinded for the study groups.

**Histopathologic evaluation**

The globes were separately numbered, fixed in a solution of 10% formaldehyde, and prepared for the histological examination. The tissues were embedded in paraffin wax, sections with 4-6 micrometers thickness were obtained, mounted on slides and stained with Hematoxyline and Eosine for routine light microscopy. The slides were histologically investigated by the same pathologist, masked as to the treatment group. Multiple sections from paraffin blocks were performed to obtain the desired lens section with maximal antero-posterior diameter, so that the section would pass from the middle of the lens, not periphery.

**Results and Discussion**

Histopathological examinations of the lenses by light microscope in control group disclosed normal findings such as a regularly shaped single layer of anterior cuboidal epithelial cells and regularly arranged cortical and nuclear lens fibers and peripherally located lens fiber nuclei, and a clear posterior lens capsule without any lining epithelial cells behind the equator (Fig. 1).

As pathologic findings four histopathological changes were demonstrated. Irregularly oriented lens fibers with liquefaction and presence of a swollen fiber cells both in the anterior and posterior cortices named as “lens fiber degeneration and destruction” (Fig. 2). Prominent epithelial cells lining the posterior lens capsule behind the equator with retention of nuclei into central region named as “posterior cataract” (Fig. 3).

Some of the lenses were morphologically immature and defective, suggesting an arrest during ocular embryogenesis at the lens vesicle stages named “immature and defective lens” (Fig. 4). Vacuoles in the cortical lens named as “vacuolar changes” (Fig. 5).

Cataractous effects were seen in 86.7% in Group I (26 of 30 newborn rats), in 73.3% in Group II (22 of 30 rats) and only nine of 30 rats in the control saline injected. The increased cataractogenic effect of the two additives were statistically significant when compared to the control group (Chi2:31.535, p<0.05).
Fig. 1. The normal findings; lens has clear posterior lens capsule (Original magnification X 40, Hematoxyline and Eosine staining).

Fig. 2. Liquefaction and swollen lens fibers (Original magnification X 200, Hematoxyline and Eosine staining).

The results for all groups and histopathological changes were given on Table 1. There was no difference between the effects of 2A5NP and 2NPPD additives (Chi2:1.834, p>0.05) (Table 2).

This experimental animal study is the first investigating maternal hair dye exposure during pregnancy which demonstrated serious deleterious effect on embrio-fetal lenticular development in neonatal rats. In our study, hair dye additives, 2A5NP and 2NPPD, were given subcutaneously during the organogenesis period (9-20 days). There are studies demonstrating the absorption of such hair dye additives from the skin in which the metabolite of this dye (P-Toliendiamine) had been shown in the human urine (6). Marks et al (7) showed in-
creased number of malformed newborn rats born to mother albino rats which were administered 2NPPD 150 mg/kg/day subcutaneously between 7th and 15th days of gestation in their experimental study. Moreover maternal exposure during 7th and 15th days of gestation of such known teratogenic agents like caffeine has been shown to cause nuclear and lenticular cataract in newborn rats (8). Histologically, the anterior subcapsular epithelial monolayer normally terminates at
Fig. 5. Vacuoles in cortical lens (Original magnification X 400, Hematoxyline and Eosine staining).

### TABLE 1

<table>
<thead>
<tr>
<th>Histopathological changes of crystalline lens</th>
<th>Group I 100 mg/kg 2A5NP</th>
<th>Group II 150 mg/kg 2NPPD</th>
<th>Group III Control Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degenerative and destructive changes of cortical area</td>
<td>N: 4, %: 13.3</td>
<td>N: 6, %: 20.0</td>
<td>N: 3, %: 10.0</td>
</tr>
<tr>
<td>Posterior cataract</td>
<td>N: 7, %: 23.3</td>
<td>N: 3, %: 10.0</td>
<td>N: 2, %: 6.7</td>
</tr>
<tr>
<td>Vacuolar change of cortical area</td>
<td>N: 7, %: 23.3</td>
<td>N: 5, %: 16.7</td>
<td>N: 1, %: 3.3</td>
</tr>
<tr>
<td>Immature and defective lens</td>
<td>N: 8, %: 26.7</td>
<td>N: 8, %: 26.7</td>
<td>N: 3, %: 10.0</td>
</tr>
<tr>
<td>No cataract</td>
<td>N: 4, %: 13.3</td>
<td>N: 8, %: 26.7</td>
<td>N: 21, %: 70.0</td>
</tr>
<tr>
<td>Total</td>
<td>N: 30, %: 100</td>
<td>N: 30, %: 100</td>
<td>N: 30, %: 100</td>
</tr>
</tbody>
</table>

The lens equator, and thin posterior lens capsule is normally clear with no lining epithelial cells in Hematoxyline and Eosine stained preparations. Posterior subcapsular cataract results from the migration of the lens epithelial cells over the posterior lens capsule behind the equator (9). This change was clearly observed in some of the lenses in the offspring of experimental groups. It could be suggested that lens epithelial cells might be damaged by toxic response, causing cataractous development by distribution anomalies of epithelial cells. Lens fibers become displaced towards the center of the lens, their nuclei normally disintegrate so that central area lacks nuclei. Teratogenic effect of congenital diseases like rubella is characterized by the persistence of the epithelial nuclei within the lens fibers in the deeper and central area (10). This finding was also significantly more common in lenses of experimental groups one and two compared to the control group (p<0.05). Whereas there was no significant difference in the cataractogenous effect of 2A5NP and 2NPPD administration (Table 2).

Cortical cataract results from the degeneration, destruction and liquefaction of the control lens fiber cells. In its incipient
stage, the formation of vacuoles, globules or clefts in the lens cortex is observed. Then interrupted and folded lens fibers and cortical clefts are filled by morgagian globules, degenerated lens cortex swells by osmotic effect, and total liquefaction eventually ensues. Such histopathological changes were demonstrated by Rogers and Crabowsky (11) on mirex-induced cataractogenesis in perinatal rats. In the present study these findings were considerable with marked liquefacions and destruction of the cortical lens fibers in the anterior and posterior poles. In addition, there were quite a number of irregularly oriented swollen cortical lens fibers. Therefore, these findings indicated a lens with cortical and complicated cataract. At postnatal day 20, some lenses were developmentally immature, suggesting arrested maturation at the lens vesicle stage which may have resulted from developmental abnormality with an early arrest in the lens cell differentiation (9, 10).

Jain et al (12) showed the cataractogenic effects of paraphenylenediamine in their experimental study. They applied paraphenylenediamine to albino rats and rabbits in various ways such as local instillation of drops in rats’ eyes, dyeing hairs, subconjunctival injection and intravitreal injection. Keratitis and corneal opacities were observed by local instillation, lenticular opacities were observed by dyeing skin. In that study after subconjunctival injection the dye has been detected in the anterior chamber by fluorometry. Also subconjunctival and intravitreal injections of the same dye caused posterior cortical and nuclear cataract (12).

In conclusion, our study demonstrated that use of hair dyes in pregnant rats induced histopathological lens changes without doubt. Ocular changes were observed in the litters of dams with both of the additives. These results may suggest that pregnant women should refrain from using hair dye in excess, especially during the 1st trimester. The ocular effects are needed to be assessed by further studies both on newborn and surviving litters.

REFERENCES