Reversal of ethambutol induced optic neuritis with natural honey in rabbits

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ABSTRACT

BACKGROUND: Ethambutol induced optic neuritis is common side effect in anti tubercular therapy. Bilateral progressive painless visual blurring and or decreased color perception may be noted. The present study was aimed to see the reversal of Ethambutol induced optic toxicity with natural honey.

OBJECTIVE: To study the reversal of ethambutol induced optic neuritis with natural honey in rabbits.

MATERIAL & METHODS: This experimental study was carried out on fifteen (15) rabbits between July 2013 to September 2013, divided in to three groups as A, B and C. Group A was used as negative control, Groups B (experimental) and Groups C (control) were given ethambutol in dose of 100 mg/kg/day respectively for six weeks. Group B was given honey at dose of 15 mg/kg/day for another four weeks. Groups C was used as control (no honey given) for comparison to see role of honey in reversal of ethambutol induced optic neuritis. Optic nerves were isolated, fixed in formalin and subjected to histopathological examination. The histological changes were recorded and graded according to a rating scale and analyzed with SPSS version 17.

RESULTS: Histological changes at Ethambutol dose of 100 mg/kg/day were 7±.00, where as dose of 100 mg/kg/day for six weeks followed by Natural Honey for four weeks at dose of 15mg/kg/day were 5.00±.894. At ten weeks interval the histological changes at Ethambutol dose of 100 mg/kg/day for six weeks followed by no treatment with Natural Honey for four weeks were 5.50±.548. Ethambutol toxicity was confirmed histologically in groups B and C at dose of 100mg/kg/day. Histological changes with honey (P value 0.003) and without honey (P value 0.001) showed no significant changes when compared, suggesting that reversal of optic neuritis may be spontaneous and not necessarily dependant on Natural Honey.

CONCLUSION: The present study confirmed ethambutol induced optic toxicity, however, partial reversal of toxicity was seen with no significant effect of honey. It is concluded that the recovery from optic neuritis may be spontaneous due to withdrawal of Ethambutol.

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INTRODUCTION:

Ethambutol hydrochloride is one of the main antituberculous drugs. Ocular toxicity has been recorded since its first use in treatment of tuberculosis in 1960s[1], manifesting as optic neuritis in affected individuals[2]. Ethambutol has been known to cause retrobulbar neuritis (axial fibres or periaxialfibres). Both axial and periaxial fibers are also shown to be affected[3].

Ehambutol produces degeneration and loss of optic nerve axons having diameter of 1 µm or less.[4] Different experimental animals, when treated with toxic doses of ethambutol, revealed multiple cystic lesions, mild demyelination, axonal fragmentation, myelin like structure in the axoplasm along with central necrosis and inflammatory changes in the optic nerve.[5] Some studies show that effect is actually on retinal ganglion neurons in rodents[6].Ethambutol has been reported to induce toxic ocular effects producing bulbar and retrobulbar neuritis, manifested as painless, symmetrical and progressive loss of vision, central or caecocentral scotoma and dyschromatopsia.[7,8,9]

Affected patients suffer from bilateral progressive painless visual blurring. Patients also complain of decreased color perception. Central vision loss has also been reported in some cases. It is believed that ocular toxicity is dose and duration related, and is largely reversible on drug discontinuation. It has been shown that 18% of patients suffer from retrobulbar neuritis who receive ethambutol >35 mg/kg/day, 5-6% retrobulbar neurtis with 25 mg/kg/day and <1% retrobulbar neuritis with 15mg/kg/day of ethambutol hydrochloride for more than two months. There is no "safe dosage" for ethambutol, toxicity can be caused by as low dosage as 12.3 mg/kg (Choi and Hwang, 1997; Gerald *et al.,* 2010[9,10].

It is believed that ethambutol toxicity is reversible but cases have been reported which caused permanent visual impairment.[11]

Oxygen is essential for life but when it is metabolized, the free radicals are produced which causes disruption of cells and as a result there is aging process. Anti oxidants which are present in body or those which are taken as food, neutralize

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the free radicals[12]. A lot of research is going on to see which mechanism causes ocular neurotoxic effect, but exact mechanism is unknown until the recent times. Blockage of mitochondrial oxidative phosphorylation and chronic increase of reactive oxygen species have been reported to be the underlying cause of both inherited and acquired types of optic neuropathy including ethambutol toxicity . Ethambutol chelates metals such as copper and iron, which are essential for complex IV and I function, respectively in oxidative phosphorylation[13,14].

Optic neuritis is believed to be due to oxidative stress which results in imbalance between free radical production and antioxidant levels in the body[15,16]. Oxidative stress is an "imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage by various oxygen and nitrogen reactive species". It is caused by increased production and/or reduced removal of reactive species by the antioxidant defenses[17].

Cell membranes lipids are vulnerable to oxidative damage by various optic toxins in the form of industrial chemicals, atmospheric pollutants, metals, pesticides, therapeutic drugs, abused drugs, and household chemicals.[18]

Natural honey has abundant antioxidants in it which can act as free radical scavengers. They can neutralize or decrease the formation of free radicals (Kishore, *et al.,* 2011)[19]. The objective of this study is to see the effect of honey which is full of antioxidants on reversal optic neuritis caused by ehambutol.

MATERIAL AND METHODS:

The study was conducted on fifteen rabbits about 1.5 to 2.5 kg in weight in the animal house of Saidu Medical College Swat. The rabbits were divided into group A(3 rabbits), B and C (6 rabbits each). Group A served as negative control group, while group B and C served as experimental and positive control groups. Each group was kept in a separate compartment under controlled conditions of temperature 20±0.5 C^0 and humidity (50 ±10%) and 12 hours light and dark cycle. They were given standardized diet and water ad libitum. Group A served as negative control (No drug or honey given). Group B and C were treated orally with ethambutol 100 mg / kg /day for 6 weeks followed by sacrifice of 3 rabbits from each group. The remaining 3 rabbits in group B were given natural honey orally in dose of 15mg/kg/day for another 4 weeks, while group C served as control for comparison (No honey given). Finally all the rabbits in group B and C were sacrificed. Both optic nerves were harvested and removed as a single piece along with the eye balls. Tissue processing was carried out for histological examination. Staining with hematoxylin and eosin was done in usual way. The slide preparations were examined under the light microscope at X40 magnification and observations were recorded. The different histological changes were recorded and graded according to a rating scale.

Means of histopathological changes in each slide were computed. All data were expressed as means ± standard deviation. Paired t-test was applied to examine the differences between various experimental and control groups, the differences were examined for statistical significance using the same paired t-test. $P < .05$ was considered statistically significant. SPSS version 17 was used for all analyses.

RESULTS:

The slides were prepared and checked under the microscope and the histopathological changes were rated according to the scale as explained in the table below. The changes recorded at 6 and 10 weeks were put in SPSS version 17 and the results calculated.

The histopathological changes were divided into two categories i.e. Category A and Category B. Category A changes are based on nuclear alignment of schwann cells, which are indirectly suggestive of demyelination. There are three subgroups in this category.

Group 1: Nuclear alignment of Schwann cells along the nerve fiber is intact with sparse clumping of nuclei at some places.

Group 2: Nuclear alignment of Schwann cells along the nerve fiber is intact with frequent clumping.

Group 3: Nuclear alignment of Schwann cells along the nerve fiber is lost with large clumping of nuclei.

Category B changes are based on loss of eosinophilia (loss of protein content) in cytoplasm evidenced by vacuolation.

Group 1: Mild loss of eosinophilia with appearance of small vacuoles in the cytoplasm.

Group 2: Gross loss of eosinophilia with appearance of large vacuoles in the cytoplasm.

Table 1:Rating scale of histological changes (assessment tool)

Category A Changes :	Rating
No changes	1
Group 1	2
Group 2	3
Group 3	4
Category B Changes :	**Rating**
No Changes	1
Group 1	2
Group 2	3

At six weeks the histological changes in group B and C at Ethambutol dose of 100 mg/kg/day were 7±.00 (Table 2). At ten weeks interval the histological changes at Ethambutol dose of 100 mg/kg/day for six weeks followed by Natural Honey for four weeks at dose of 15mg/kg/day were 5.00±.894 (Table 3). At ten weeks interval the histological changes at Ethambutol dose of 100 mg/kg/day for six weeks followed by no treatment with Natural Honey for four weeks were 5.50±.548 (Table 3). Ethambutol toxicity was confirmed histologically in groups B and C at dose of 100mg/kg/day. After giving honey for 4 weeks to group B, we found that there was significant improvement in histological changes at 10 weeks (P value 0.003) (table 4). On the other hand, toxicity reversal in the control group C (No Natural Honey for four weeks) was also significant at 10 weeks (P value 0.001) (table 4). The statistical difference between experimental and control groups was not significant (P >0.05) (table 5), suggesting that reversal of optic neuritis may be spontaneous and not necessarily dependant on Natural Honey.

DISCUSSION:

In the current study, toxic vacuoles were observed in all preparations obtained from experimental B and Control C groups (7±.00) at 6 weeks. These results of our study support the earlier observations in previous studies.[20,21] These vacuoles indicate axonal degeneration induced by ethambutol mediated increased Ca++ influx activating certain intracellular enzymes causing cell membrane damage, digestion of cytoskeletal

Table 2: Histological changes in B and C at 6 weeks

Groups	Dose	Histological changes according to rating scale						Mean	S.D
B	100mg/kg/day	7	7	7	7	7	7	7	.000
C	100mg/kg/day	7	7	7	7	7	7	7	.000

Table 3: Histological changes in B and C groups at 10 weeks interval (n=6)

Groups	Dose	Histological changes according to rating scale						Mean	S.D
B	100mg/kg/day+honey	5	6	4	6	4	5	5.00	.849
C	100mg/kg/day+no honey(control)	6	6	5	6	5	5	5.50	.548

Table 4: Toxicity due to ethambutol at 6 weeks vs 10 weeks

Pairs	P value	Significance
Pair I Group B 6 weeks vs. Group B 10 weeks (ethambutol100mg/kg/day and honey 15mg/kg/day)	.001	<.05 Significant
Pair II Group C 6 weeks vs. Group C 10 weeks (ethambutol 100mg/kg/day + no honey)	.003	<.05 Significant

Table 5: Comparison between honey and no honey groups at 10 weeks

Pairs	P value	significance
Pair 1 Group B 10 weeks vs. Group C 10 weeks (honey vs. no honey groups)	.076	>.05 Not Significant

proteins and ATP depletion, which results in failure of Na+/K+ pump leading to cellular swelling, anaerobic glycolysis, impaired protein synthesis and accumulation of lipids inside the cell that microscopically appear like vacuoles[22]. Other possible mechanism stipulated the metabolites of ethambutol forming a complex with Zn and Cu which enters the axons and enhances the axonal dilatations (vacuolation) process[22]. Demyelination, and myelin like structure in the axoplasm were observed in our study which are comparable to previous studies on different animals[20,21]. The toxic changes in group B markedly decreased at 10 weeks when treated with honey for 4 weeks(5.00±.894). This reversal of changes was statistically significant (p .001). Similarly toxic changes were also reversed in control group C (5.50±.548), in which no honey was given. The difference in changes was statistically significant (p .003). The difference in the honey group B and non honey group C was statistically not significant (p value .076). These figures show that the honey did reverse the changes more than the non honey group, but the difference is not significant. The protective effect of honey on ethambutol induced changes in the optic nerve has not been studied before so that we could compare our results with other studies. However, honey has been used with best results in the treatment many diseases. Honey has significant prophylactic and therapeutic value against antitubercular drugs induced hepatotoxicity[23]. Honey, a natural product formed from nectar by honeybees, has been shown to exert several health-beneficial effects such as gastroprotective[24], hepatoprotective[25], reproductive[26], hypoglycemic[27], antioxidant[28], antihypertensive[29], antibacterial[30], anti-fungal[31] and anti-inflammatory[32] effects.

Propolis, also known as "*bee glue*," has been used in folk remedy for several ailments and various pharmacological properties such as anti-inflammatory[33], antimicrobial[34], antioxidant[35], immunostimulant[36], antitumor[37], neuroprotective[38], and hepatoprotective activity[39] have been reported in different studies.

CONCLUSION:

In our study, honey has not proved effective in reversal of ethambutol induced optic neuritis. The reversal seems to be spontaneous due to withdrawal of the drug.

Recommendation : Studies with higher doses of honey are suggested. Preventive aspect of honey, also needs to be explored, so that honey can be given to patients using antituberculous drugs to avoid its side effects.

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