

pathophysiological effect of Pegensynthetic dye On Rabbit(*Oryctolagus cuniculus*) Renal and liver functions

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Abstract :

Pegen is a type of commercial synthetic dye used for hair and skin ,it is toxic chemical compound which contains Paraphenylenediamine (PPD) mostlyharmful when the exporters were repeated either by inhalation or absorption and affected the physiology of human and animals systems,Moreover,this study was constructed to find physiological effects on blood parameters ,liver and the kidneys of the rabbits(*Oryctolagus cuniculus*). Thirty sixexperimental laboratory animalswere used to fulfil this study and divided into three groups,each of 12 animals, first one is control, while the second for application of natural henna and the third one for Pegen synthetic dye at intervals of 10 days for six months .

Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) were used for defecting and determining the concentration of PPD in Pegen(0.187mg/l). Blood samples were collected every two months for tests of kidney and liver functions and haematological indicesthe red

blood counts were measured by Sysmex-KX which recorded low values of $3.92 \times 10^6/\text{ul}$ compared to control which found to be $5.16 \times 10^6/\text{ul}$. The results of haemoglobin concentration (9.00g/dl) similarly were showed significant slight decrease compared to the control for 11.42mg/dl whereas leukocyte count with value $6.89 \times 10^3/\text{ul}$ were record significant increase to the value $9.55 \times 10^3/\text{ul}$. Also, liver test for GOT GPT ALT AST ALP/ul, T.P, Alb and Bili mg/dl of values 105.80, 86.40, 86.60, 350.74, 6.69, 5.60, 6.17 and 1.29 were showed abnormality compared to control values of 37.40, 19.70, 67.46, 297.20, 5.05, 7.45, 3.42 and 0.42 respectively.

On other hand, kidney function tests were found to be abnormal in those who used Pegen such as blood urea, Creatinine Potassium, and Sodium for values 1.81, 57.43, 6.19 and 120.50 compared to the natural henna values of 0.64, 29.60, 5.22 and 142.15 respectively ($p < 0.05$).

1. Introduction

The skin is the main target of allergic reaction to PPD where, Humans are systematically exposed to acetylated derivatives e.g. Monodactyl-PPD (MAPPD), which in turn is acetylated to N-Diacetyl-PPD (DAPPD), is competitively inhibited in presence of p-aminobenzoic acid (Yokawakudo, et.al., 1999, frédérque, et.al., 2007). However, acute exposure of high level of Paraphenylenediamine may cause severe dermatitis, asthma attacks, born) (Chakraborty et. al, 1977). Chospam and inflammation of pharynx and larynx. The effects are more usually as result of sensitivity reactions. Moreover, Quinine diamine is thought to be responsible for sensitization property of PPD. Breathing of dust of PPD quinine is found in respiratory tracts (Jacobs, 1949.)

Ocular exposure to PPD causes mucous membrane irritation and hypersensitivity to it (<http://www.hennaforhair.com/ppd/>). As with other routes of exposure, hypersensitivity following

previous exposures to PPD includes chronic dermal exposure lethargy, myalgia, purplish, discoloration of gums and teeth anorexia, gastro-intestinal disturbance, liver and spleen enlargement, sub-acute atrophy of the liver, jaundice, chronic renal failure, progressive neurological symptoms and coma have all been attribute to chronic exposure to PPD (Yagietal,1991).PPD is responsible for most complications reported after henna tattoos: Localized or generalized contact dermatitis, hypertrophic or keloid scars and temporary or permanent hyper or hypo pigmentation more rarely type I hypersensitivity reactions with potential lethal outcomes have been reported (Nicolas, et al., 2008). Moreover, in patch tests on 691 patches with allergic dermatitis during a 17 month period proved that 6% of patients responded to PPD. Ng-SK, (1990) has considered PPD as one of contact allergens that produces different types of reactions such as allergic contact dermatitis (ACD) and immunological contact urticaria (ICU), where Nethercott et al (1986) found that 18 cases of hand dermatitis in hair dressers were seen over 5 years period and diagnosed as contact allergy due to PPD. Studies of intra cutaneous sensitization of guinea pigs using PPD hydrochloride, quinhydrone and benzoquinone revealed that benzoquinone formation plays an important role in the allergic reaction of PPD (Rajaka & Blohm, 1970). They found that small amount of the dye can penetrate the skin and cutaneous absorption and ingestion profuse severe local reactions and systemic effects (Gleason, et. al., 1963). In addition to PPD is a potent skin sensitizer; it can cause angio-neurotic edema, collapse, and renal failure in severe cases (Abdulla & Dasvion, 1996). Severe cases of immediate type hypersensitivity to PPD described in which the patients developed severe edema, irritation of the eyes and face and also difficulty in breathing (Calman et al 1967). In another study PPD was found to be the most common sensitized in

bred guinea-pigs were prepared and cross reaction of PPD was examined using cultural epidermal cells as target, the cytosolic effect of PPD was very high in 107 cases of facial contact dermatitis, test with TRO-LAB European standard allergens (ESAs) revealed that 57% had positive reaction. The most frequent contact allergens were PPD hydrochloride 16% (Zhao & Fan, 1991). Overall objective of this study to highlight the toxicity of synthetic dyes black henna or stone dye compared to natural henna. With the following specific objectives.

To investigate the toxicity of synthetic dye Pegen added to henna, compared to natural henna.

To study the physiological effects of Pegen on the Rabbits according to exposure time

2. Material and Methods: 2.1 Laboratory animals:

Local strain of rabbits of 7 pregnant females and one male were purchased from special farm located near Bahri local market. They were transferred and reared in special constructed house at the Natural History Museum with a good health condition. Beside the presence of burrows for breeding they were mainly fed on sorghum, carrot and carrot leaves. After the appearance of first progeny, were reared until reached the weight (900-1000g). The experiments were performed. Thirty-six experimental laboratory animals were divided into 3 groups, each of 12 rabbits. One group was served as control negative group, whereas two other groups (24) were dyed using about 60 mg of paste of natural henna and the synthetic dye Pegen respectively. This process was done on an area (3×5cm) on the backside of rabbit. The tested area was covered for protection. This process lasted about one hour and was repeated with intervals of 10 days during a period of 2, 4 and 6 months. During this period blood sampling is taken. For haematological and biochemical examinations.

2.2 chemical analysis

2.2.1.1 TLC analysis (Stahi, 1969)

TLC analysis was conducted at The International Research Centre to detect the presence of PPD in natural and pegen, using Soxhlet extractor apparatus (electro –mag) Gallenkamp, U.

2.2.1.2 Preparation and TLC analysis of dyes samples:

0.2g of natural dyes and pegen were extracted using Gallenkamp, U.K, and dissolved in 80% chloroform and stored till being used. 2 drops from each sample were spotted on the TLC plate.

2.2.1.3 Preparation of the plates:

30mg of silica gel were shaken with 60 ml distilled water for 2min using 250ml stoppered conical flask. The slurry was spread on plane making 0.25mm thickness on glass 20× 20 cm plates. The coated plates were then allowed to dry at room temperature and then activated at 105 c for one hour. The hot plates were allowed to cool and stored until use.

2.2.1.4 Preparation of 3 solvent solutions for TLC of dyes samples

The three solvent systems were prepared by mixing Butanol, acetic acid and water with 40, 10 and 50%, hexane and acetone with 30, 90 % and acetone, ammonia 28% and chloroform with ratios 40, 2 and 40% respectively.

2.2.2 HPLC Analysis

High performance liquid chromatography (HPLC) was done at central Lab. for Pegen to find the concentration of PPD in each dye sample, compared to the standard. The same analysis was used to check the concentration of PPD in 10 urine samples of henna artists (Hananat) (B) compared to control urine sample using (SHEMADZU), Software CLASS-VP Data system

2.2.2.1 Preparation of synthetic dye samples for HPLC:

Stock concentration of Pegen was prepared by accurately weighing 50 mg of synthetic dye using sensitive balance into a 25-ml volumetric flask. Initially, dissolve the Pegen with about 20 ml of 10 N Sulphuric acid. After totally dissolved dilute to the mark with additional 10 N Sulphuric acids and thoroughly mix the solution. They were kept in a refrigerator until being used.

3. RESULTS

3.1 TLC Analysis Results:

TLC Plate (1a, 1b) shown two dark spots for synthetic dye Pegen and the standard at different R_f values of 3.3/16 and 8/16 for two solvent systems (A) is (Hexane: Acetone) with ratio 30:90 respectively, (B) another solvent is used by spraying with potassium dichromate using Chloroform: Acetone: Ammonia.

Plate (1a)



A

Plate (1b)



B

3.2 HPLC Analysis Resultsof Pegen

The figures (1a,1b) shown the chromatograms of the PPD in hair dyes used by Sudanese women. The retention time for standard is 1.600-1.611 with concentration 0.200. Retentiontime of PPD in Pegen showed the chromatogram as standard retentiontime whereas the concentration was found 0.187mg.

Table (1) Percentages between the synthetic dyes Pegen and Standard PPD in 2.5 mg \ l

Sample	Concentration	Percentage
Standard Paraphenylenediamine	2.450	97%
Pegen dye	0.187	5.88%

Figures (a1, a2) shown the chromatograms of the PPD in hair dyes Pegen

Figure (a1)Figure (a2)

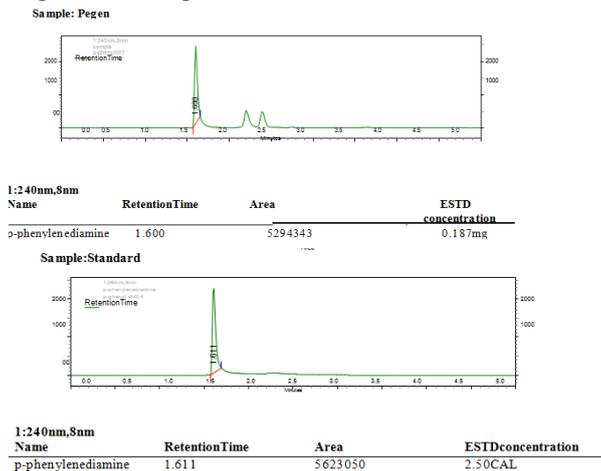


Table (2a) Haematological changes in the dyed rabbit blood during different interval of time

Interval of time (months)	Animal group	Haematological parameters			
		Hb (g/dl)	RBCs×10 ⁶ /ul	TWBCs ×10 ³ /ul	MCH (pg)
After 2 months	Control	11.42±0.2 ^a	5.16±0.04 ^a	6.89±0.28 ^a	22.13±0.35 ^a
	Nat.Henna	11.35±0.3 ^a	5.27±0.04 ^a	7.72±0.27 ^b	21.50±0.30 ^a
	Pegen	10.18±0.3 ^b	4.55±0.24 ^b	8.90±0.39 ^c	25.52±0.23 ^b
After 4 months	Control	11.42±0.2 ^a	5.16±0.04 ^a	6.13±0.28 ^a	22.13±0.35 ^a
	Nat.Henna	11.30±0.3 ^a	5.32±0.04 ^a	6.72±0.25 ^b	22.17±0.34 ^a
	Pegen	9.72±0.3 ^b	4.35±0.14 ^b	7.91±0.28 ^b	27.08±0.35 ^b
After 6 months	Control	11.42±0.2 ^a	5.16±0.04 ^a	6.13±0.24 ^a	22.13±0.32 ^a
	Nat.Henna	11.52±0.3 ^b	5.55±0.04 ^b	7.92±0.28 ^b	22.24±0.35 ^a
	Pegen	9.00±0.3 ^c	3.92±0.23 ^c	9.55±0.33 ^c	27.91±0.37 ^c

Nat. =Natural, a,b,c. means the mean with different superscript in the row column are significantly different at P<05

Table (2b) Haematological changes in the dyed rabbit blood during different interval of time

Interval of time (months)	Animal group	Haematological parameters			
		MCV/Fl	MCHC (g/dl)	Lymph%	Mix%
After 2 months	Control	76.87±0.36 ^a	31.43±0.40 ^a	70.65±0.3	10.65±0.32
	Nat..He	76.74±0.32 ^a	31.12±0.40 ^a	69.34±0.3	9.69±0.32 ^b
	Pegen	78.92±0.22 ^b	32.17±0.43	79.62±0.3	16.24±0.22
After 4 months	Control	76.87±0.36 ^a	31.43±0.40 ^a	70.65±0.3	10.65±0.31
	Nat.He	77.12±0.33 ^b	31.32±0.40	70.58±0.3	10.42±0.32
	Pegen	81.01±0.11 ^c	35.30±0.41 ^c	80.75±0.3	18.04±0.30
After 6 months	Control	76.87±0.36 ^a	31.43±0.40 ^a	70.65±0.2	10.65±0.33
	Nat.He	76.71±0.32 ^a	31.42±0.44	70.01±0.3	11.02±0.32
	Pegen	83.51±0.14 ^c	37.90±0.34 ^c	80.52±0.33 ^c	20.11±0.23 ^c

Nat. =Natural, a,b,c. means the mean with different superscript in the row column are significantly different at P<05

Table (2a,b) shows the changes of the blood parameters according to application of natural and synthetic dye on the rabbits.

The effect of Pegen dye on Hb, RBCs counts the mean cell hemoglobin (MCH), the mean cell volume (MCV) similar to the mean cell hemoglobin concentration (MCHC) blood parameter

showed clear significant decrease when compared with control group (11.42g/dl) ($P < 0.05$).

However, there is no significant difference with natural dye henna 11.39g/dl ($P < 0.05$). On the other hand, TWBCs counts of the tested groups with Pegen which includes Lymphocytes percentages, basophil and eosinophil (mix) percentages showed significant increase ($P < 0.05$) compared to the control (6.39×10^3 /ul).

Table (3) Liver function test of dyed blood rabbits

Interval of time (months)	Animal group	Haematological parameters							
		GOT /ul	GPT /ul	ALT/ UI	AST/ UI	ALP /ul	T.P g/dl	Alb. mg/dl	Bili mg/dl
After 2 months	Control	37.40±0.06 ^a	19.70±0.03 ^a	67.46±0.03 ^a	297.20±0.96 ^a	5.05±0.03 ^a	7.67±0.23 ^a	3.42±0.89 ^a	0.42±0.02 ^a
	Nat.Henna	38.67±0.03 ^a	18.91±0.02 ^a	68.51±0.00 ^a	294.76±0.92 ^a	5.01±0.00 ^a	7.59±0.22 ^a	3.45±0.88 ^a	0.40±0.02 ^a
	Pegen	98.31±0.05 ^c	71.23±0.04 ^b	75.51±0.02 ^b	313.23±0.90 ^b	5.43±0.05 ^a	6.42±0.20 ^b	4.36±0.89 ^b	0.53±0.00 ^b
After 4 months	Control	37.40±0.06 ^a	19.70±0.03 ^a	67.46±0.03 ^a	297.20±0.96 ^a	5.05±0.03 ^a	7.45±0.23 ^a	3.42±0.86 ^a	0.42±0.02 ^a
	Nat.Henna	38.44±0.02 ^b	19.71±0.03 ^a	68.72±0.01 ^b	299.21±0.93 ^a	5.31±0.01 ^a	7.59±0.26 ^a	3.61±0.88 ^a	0.42±0.01 ^a
	Pegen	100.21±0.05 ^c	74.41±0.04 ^b	81.21±0.04 ^b	335.21±0.91 ^c	6.71±0.04 ^b	5.31±0.21 ^b	5.50±0.85 ^b	0.73±0.02 ^b
After 6 months	Control	37.40±0.06 ^a	19.70±0.03 ^a	67.46±0.03 ^a	297.20±0.96 ^a	5.05±0.03 ^a	7.45±0.23 ^a	3.42±0.89 ^a	0.42±0.02 ^a
	Nat.Henna	38.36±0.06 ^b	18.60±0.00 ^a	68.42±0.00 ^a	298.35±0.96 ^b	5.02±0.03 ^a	7.61±0.21 ^a	3.57±0.87 ^b	0.43±0.02 ^a
	Pegen	105.80±0.04 ^c	86.40±0.02 ^b	86.60±0.03 ^c	350.74±0.92 ^c	6.69±0.00 ^b	5.60±0.25 ^c	6.17±0.88 ^c	1.29±0.00 ^c

Nat. =Natural a,b,c. means the mean with different superscript in the row column are significantly different at P<05T.Key: P=Total protein Alb=albumin Bili =bilirubi

The changes of the liver parameters (Total protein, albumin, bilirubin, creatinine, plasma enzymes and plasma elements) according to application of synthetic dye. The effect of, Pegen were highly significant ($P<0.01$ when compared to the control group) the calcium level showed no significant difference.

the effects of Pegen of activity of Galutamicoxaloacetate transaminase Galutamic pyruvate transaminase activity level, Alanine-amino-transferase level Asparteamino-trasferase alkaline Phosphaliquid and showed significant increase ($P<0.05$) as compared to the control

Table (4) Renal test of dyed blood rabbits:

Interval of time (months)	Animal group	Haematological parameters				
		Creatinine mg/dl	Blood urea mg/dl \pm 0.	Serum k. mmol/l	Serum Na. mmol/l	Serum Ca mmol/l
After 2 months	Control	0.51 \pm 0.02 ^a	29.67 \pm 0.39 ^a	5.74 \pm 0.04 ^a	140.42 \pm 0.54 ^a	9.67 \pm 0.23 ^a
	Nat.Henna	0.61 \pm 0.02 ^b	29.60 \pm 0.33 ^a	5.15 \pm 0.04 ^a	143.24 \pm 0.55 ^a	9.71 \pm 0.23 ^a
	Pegen	1.46 \pm 0.00 ^c	40.33 \pm 0.31 ^b	6.55 \pm 0.02 ^b	136.15 \pm 0.50 ^b	9.31 \pm 0.20 ^b
After 4 months	Control	0.51 \pm 0.02 ^a	29.67 \pm 0.39 ^a	5.74 \pm 0.04 ^a	140.42 \pm 0.55 ^a	9.67 \pm 0.21 ^a
	Nat.Henna	0.64 \pm 0.01 ^b	29.60 \pm 0.39 ^a	5.22 \pm 0.03 ^a	142.15 \pm 0.58 ^a	9.50 \pm 0.2 ^a
	Pegen	1.63 \pm 0.01 ^c	54.92 \pm 0.33 ^c	6.23 \pm 0.04 ^b	129.18 \pm 0.54 ^c	7.29 \pm 0.23 ^b
After 6 months	Control	0.51 \pm 0.02 ^a	29.67 \pm 0.38 ^a	5.74 \pm 0.04 ^a	140.42 \pm 0.55 ^a	9.67 \pm 0.20 ^a
	Nat.Henna	0.64 \pm 0.02 ^b	29.66 \pm 0.33 ^a	5.13 \pm 0.02 ^a	143.11 \pm 0.51 ^b	9.63 \pm 0.23 ^b
	Pegen	1.81 \pm 0.03 ^c	57.43 \pm 0.32 ^c	6.19 \pm 0.06 ^b	120.50 \pm 0.54 ^c	8.22 \pm 0.2 ^b

Nat. =Natural ^{a,b,c} means the mean with different superscript in the row column are significantly different at $P<0.05$. Key: K=Potassium, Na=Sodium, Ca=Calcium

The change renal test parameters mainly blood urea and creatinine according to increasing exposure time, Moreover, Potassium level of the blood plasma changes of tested group with, Pegen showed significant increase ($P < 0.05$). Sodium level in the blood plasma contents of the tested group, showed significant decrease ($P < 0.05$). On the other hand, calcium measurements showed irregular effects.

4. Discussion

There was a previous research done on rats and chicken which were exposed to PPD by various routes (oral, cutaneous, subcutaneous and intramuscularly) correlated to the time and the concentrations of PPD was used, significant correlation to both factors was obtained. (Saad, 1996) in this study the toxicological effects of PPD take place through skin absorption because synthetic dyes were applied on shaving area in the of the rabbits.

In this study the effect of the duration of time exposure to PPD and the synthetic dyes used were significantly correlated with blood parameters. Similarly, the toxicity effect of PPD correlated toxicity route and with the amount of the dose (Saad, 1996).

In this study the renal parameters creatinine, blood urea, uric acid and electrolytes were found to be high affected as compared with the normal ranges and the effects correlated with duration time of exposure. Similar results, obtained by Hashim, et.al., 1984, Yagi, 1991) hence functional reserve of the kidney is large, and much damage may occur before functional impairment is evident

Recommendation:

In spite of moderate toxic effects of Pegen which is appear as abnormal result of blood, renal and liver test parameters. Up to now Sudanese women used to mixed Pegen with henna to accelerate henna dying processes and to avoid repeating henna. On the other hand, long exposure to PPD lead to precipitation of it on tissues which lead to short or long

term of physiological effects on liver, kidneys and skin. The study recommended there must be scientific awareness and acquaintance among women to choose natural henna as more safe cosmetic dye.

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