Paraphenylenediamine (PPD) is a synthetic compound not known to occur in nature. Paraphenylenediamine $C_6H_4(NH_2)_2$ is a derivative of paranitroaniline, (Brown et al, 1987). It is a colourless crystalline solid when pure but rapidly darken and turns brown on exposure to air. It has the following Chemical names:

1.4- benzenediamine, parabenzenamine, 1,4- phenylenediamine. (IARC monograph, 1977). The Chemical formula is illustrated in Fig. 1 and its molecular weight is 108, and the melting point is 145-147°C (MerckIndex, 1983).

Preparation of PPD dates back to 1878 when Ramine and Zincke prepared the compound by reducing 1,4- dinitrobenzene with tin and hydrochloric acid as stated by Prager et al (1930). It is produced commercially by reducing 1-amino-4- nitrobenzene with iron and hydrochloric acid or iron and ferrous chloride (Thittle, 1968).

PPD has been produced for commercial utilization for many years. It is believed that at least four countries produce PPD in Western Europe. About 10-100 thousand Kg
are produced annually in UK. Japan and U.S.A. also produce the same amount or more. The purity of the produced compound varies between 99% and 99.5%.

PPD is widely used as a hair dye and most of the hair dye formulations contain variable concentrations of PPD (0.2% - 3.75%). It is used in the industry of tyre cords, photographic developer, photochemical measurement and variety of antioxidants (Yagi et al., 1991).

In Sudan PPD (in its pure form 99%) is used for dying hair, but lately it became widely mixed with henna (*lawasonia alba*) to intensify the black colour produced by henna and also to reduce the time required for the whole process (El-Ansary et al., 1983). People in Sudan and some other countries has been aware of the toxic effects of PPD so it is broadly used for suicidal and homicidal purposes (Al/Karim et al., 1992). PPD has been identified as one of the constituents of home made remedy provided by local witch doctors as a pain killer (Averbukh et al, 1989).

1.2 Reaction

Paraphenylenediamine undergoes two main reactions that contribute to its biological activity.

1.2.1 Oxidation

PPD when oxidized produces quinone-dimine NH : C₆H₄ : NH (Fig.2), which may be oxidized to a complex known as Bandrowskis base.

![Fig. 2 Oxidation Reactions of PDD](image-url)
In hair dye formulations PPD is oxidized by oxygen that results from the decomposition of hydrogen peroxide to give quinone diimine. The later reacts with another constituent of hair dye paste known as coupler to give the desired colour (Fig.3), (Burnett et al., 1977).

![Fig. 3 Coupling Reaction of Quinone diimine.]

1.2.2. Deamination
Deamination has been suggested as a mode of action of PPD which results in production of aniline that may contribute in part to the toxic effects (Nott, 1924).

1.3. Mode of Action
Paraphenylene diamine is a reducing agent and is thought to be oxidized \textit{in vivo} to quinone diimine. It has a sharp penetrating odour and produces violent local irritation of the mucous membranes and skins of sensitive individuals. Quinone diimine is suggested to be responsible for the sensitization property of PPD. Breathing the dust of PPD quinone diimine is formed in the respiratory tract (Jacobs, 1949). It seems likely that production of aniline is also responsible in part to the toxic symptoms (Nott, 1924).

Lipid peroxidation is suggested to be responsible for tissue damage associated with PPD poisoning. This can be demonstrated histopathologically and biochemically by changes in cytoplasmic enzymes (Mathur \textit{et al.}, 1990). PPD undergoes rapid autoxidation in the culture medium at low concentration (upto 10\(\mu\)g/ml) and with low exposure time (30 min). It enhances keratinocyte proliferation. At high concentration
and longer exposure time it produces cell stasis and toxicity. These effects together with the enhanced lipid peroxidation, can be ascribed to the production of supreroxide and hydrogen peroxide by the autoxidation of PPD. At non-cytotoxic concentration PPD induces intercellular adhesion molecule-1 (ICAM-1) expression on the keratinocytes. These results were consistent with the view that oxidative stress may be an essential part of the pre-immunological phase in the induction of the allergic contact dermatitis (Picardo et al., 1992). Rhabdomyolysis is considered to be the principle mechanism underlying PPD systemic toxicity. It is in particular responsible for the renal failure observed in many cases (Bourguia et al., 1988; Saito et al., 1990).

The sensitization potential of PPD is considered to be the most toxic reaction (A/Kharim et al., 1992). PPD is top listed allergen (Burnett et al., 1977). It has been estimated that about 4% of apparently normal subjects are sensitive to PPD and 1% acutely sensitive (Martindale, 1952). It was included within allergens studied by Seidenari et al. (1990). They showed erythematous urticarial papules, plaques and target lesions (erythema multiform like eruptions). They occur as a result of allergic contact dermatitis which generally presents itself as an eczematous eruption. It was noticed by erythema multiform concurrently with allergic contact dermatitis. Patch testing revealed sensitivity to PPD. Nethercott et al. (1986) found that 18 cases of hand dermatitis in hairdressers was seen over 5 years period and diagnosed as contact allergy due to PPD. Studies of intracutaneous 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 et al., 1970). In patch tests on 691 patches with allergic dermatoses during a 17 month period proved that 6% of the 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 immunologic contact-urticaria (ICU). ICU is an immunoglobulin E(IgE)-mediated mechanism. When 230 patients with occupational dermatitis in metallurgic industry were studied, PPD was found to be one of the materials that produce such effect (Alomar et al., 1985). In another study, 307 patients showed signs of contact dermatitis and PPD was found to be the most common sensitizer (Matsukubo,
When PPD specific lymphocytes from sensitized inbred guinea-pigs were prepared and cross-reaction of PPD was examined using cultural epidermal cells as a target, the cytolytic effect of PPD was very high (Shigematsu et al., 1988). In 107 cases of facial contact dermatitis, routine Finn Chamber epicutaneous test with TROLAB European standard allergens (ESAS) revealed that 57% had positive reaction. The most frequent contact allergens were PPD hydrochloride 16% (Zhao et al., 1991).

It was noticed that PPD is of high sensitization potential and produces other toxicological characteristics. However the incidence of these effects by hair dye formulations is infrequent and usually of mild occurrence. This may be due to the fact that the hair dye contains less than 3% PPD and the rest is the oxidizing material (Hydrogen peroxide) and the coupler. The half life of quinone diimine, which is formed as a result of oxidation by hydrogen peroxide during application, is just few milliseconds and its concentration never reaches a detectable level (Kiese et al., 1968). The end product of the reaction between quinone diimine and the coupler appears to be unreactive. This fact is demonstrated by recent experiments in which 20 people with strong positive patch test reaction to PPD were challenged with hair that has been dyed 24 hours previously with PPD containing system. None of them showed any reaction to the dyed hair (Reiss, 1974). However the dermatological properties have been studied for more than 50 years, but systemic toxicity was discovered recently. Burentt et al (1977) found that small amount of the dye can penetrate the skin. Cutaneous absorption and ingestion produce severe local reactions and systemic effects (Gleason et al., 1963). Investigations performed in cases admitted to hospitals had shown significant histopathological and biochemical changes. In the case reported by Suliman et al (1983), the cardiovascular, respiratory, alimentary and nervous systems were found to be normal. However the patient was anuric for four days. There was an increase in the plasma urea concentration, it was 25 mmol/L at first then increased to 108 mmol/L on the fourth day of admission and gradually decreased to normal after five weeks. Potassium level increased from 5 to 7 mmol/L but sodium was within the normal limits. Creatinine clearance was 74 ml/min. The erythrocytes sedimentation rate 48 h after admission was 80 mm. However the haemoglobin concentration and white blood cells...
(WBC) count were normal. Renal biopsy performed a week after admission showed histological picture of acute tubular necrosis. Saito et al (1990) reported a case of PPD poisoning in which the following changes were observed: high level of creatine phosphokinase (CPK), lactate dehydrogenase (LDH), glutamic oxaloacetate transaminase (GOT), glutamic pyruvate transaminase (GPT) and leukocytosis. Electrocardiogram (ECG) showed mild depression. Urine of the patient diminished and was dark brown. Renal collecting ductules and distal tubules were occluded by dark brownish myoglobin casts and its epithelium massively necrotised. Skeletal muscles showed scattered coagulation necrosis and were partially associated with inflammatory cell infiltration. Averbukh et al (1989) reported a case of PPD poisoning in which there was an increase in the level of urea, creatinine, sodium, calcium, potassium, uric acid, CPK, LDH, GOT and GPT. In the case reported by Baud et al (1983) there was an increase in the level of blood urea, creatinine, potassium, total proteins and calcium. Rhabdomyolysis was looked for because of the hard painful oedema, acute renal failure, metabolic acidosis and dark brown urine. Muscle biopsy 24 h after admission revealed massive muscle necrosis. Investigations performed by Yagi et al (1991) revealed that there was an increase in the level of blood urea, albuminuria with haematuria in 30% of the cases reported. Liver function was within the normal limits. Investigations performed by Brown et al (1987) on chronic cases of PPD poisoning showed an increase in the level of urea (129 mg/dl), creatinine (7.7 mg/dl) and haemoglobin (107 g/l). However the other case had showed higher level of urea and creatinine. Biopsy of the skin lesions showed allergic vasculitis and percutaneous renal biopsy showed crescentic glomerulonephritis. Post-mortem examination showed no evidence of healed vasculitis. Both kidneys were extremely small and there was considereable hyalinisation of the glomeruli. Many tubules were distended with colloid casts and there was fine fibrosis with infiltrate lymphocytes in the interstitial tissues. The interlobar and arcuate arteries showed thickening of their walls but there was no evidence of arthritis. In the other cases, biopsy of the lesions showed allergic vasculitis, and the development of ventricular fibrillation. Post-mortem examinations showed occlusion of the right coronary artery by haemorrhage into an atheromatous plaque and healed posterior wall
myocardial infarctions. The lungs were congested and multiple white nodules were noticed throughout. Their histopathology showed granulomatous inflammatory process and acute inflammatory infiltrate in the vascular channels and fibrinoid necrosis was noticed and was consistent with Wegener's granulomatosis. Both kidneys were enlarged and pale with histological and immunological evidence of crescentic glomerulonephritis. In the case reported by Israel et al. (1934) post-mortem examinations revealed that the heart was small, right auricle and ventricle were dilated and the myocardium was pale and flaky. The lungs were congested, the air passages contained blood stained mucus. Pale yellow nodules were protruding from the tissue. Most of the tissue has been fibrosed and instate of advanced necrosis. Kidneys enlarged and congested. The general features were of an ascending pyelonephritis. The brain showed definite acute congestion of the cortex and white matter throughout. The appearance was that of toxic encephalitis. All the organs were jaundiced.

1.4. Toxicity

The dermatological properties of PPD have been studied for more than 50 years, but the systemic toxicity was discovered more recently. The systemic toxicity associated with topical application indicated absorption of small amounts of some hair dye ingredients. That is why the European Economic Community posed regulations on the concentration of PPD in hair dye formulations which should not be more than 6% (IARC monograph 1977). In the field of industry the employees exposure to PPD should not exceed 0.1 mg/m³ in the working atmosphere in any eight hours work shift of a forty hours working week (U.S. occupational safety and health administration, 1976). The same regulations were imposed in Japan and Germany (IARC monograph, 1977).

Application of hair dye formulation for long time may results in apparent typical PPD toxicity. The major problem of PPD toxicity results from ingestion of the pure stuff (99%) for suicidal, homicidal or by mistake. However there were some cases reported as a result of topical application during hair dying using pure stuff or while practising henna.
In many countries the trade in PPD is not restricted and is widely available for home applications. In Sudan, despite the restrictions imposed on the import of PPD, it finds its way to the country through illicit channels (A/Kharim et al., 1992). Samples of commercial PPD material collected from the local markets, when analyzed quantitatively and qualitatively, were found to be of a very high purity 97% (Yagi et al., 1991).

1.4.1. Acute Toxicity

A survey of suicide attempts using PPD in the capital Khartoum (Sudan), during the years 1987-1990 revealed that there were a total of 264 cases of suicidal attempts. In 35% of them PPD was used, about 60% of the cases were 10-20 years old and 30% in the age group 21-30 years. The highest mortality rate (22.7%) was reported during year 1987 (A/Kharim et al., 1992). El-Ansary et al. (1983) stated that 24 patients were admitted to Omdurman hospital within twelve months, twelve patients took PPD intentionally and eight of them died. Death was mainly due to acute respiratory distress. Over a period of two years, a series of eighteen cases of acute PPD poisoning were reported sporadically to the accident and emergency department of Khartoum North hospital (Sudan). Two of the cases were babies about eighteen month old. The reasons for ingestion of PPD was mainly suicidal (about 70%), the remaining was accidental. The mortality rate in this series was 22%. The exact dose taken by each patient was not known, however in the serious cases, it was estimated to be about 7g (orally) to traces of PPD in mild cases (Yagi et al., 1991). Suliman et al. (1983) reported a case of fifteen year old Sudanese girl attempted suicide by drinking a solution of PPD, they also reported sixteen patients who developed toxicity of PPD after accidental, suicidal or homicidial ingestion. Twelve of the patients died within 48 h of ingestion of PPD. Saito et al. (1990) reported a case of 44 year old male (Japanese) who drank beverage containing PPD prepared for homicidial purposes and died in a course of 30 hours. It was found that the amount of PPD taken was about 30g. Averbukh et al. (1989) reported a case of 40 year male who developed typical clinical picture of PPD poisoning after taking 5g of home made remedy provided by a witch-doctor as a pain killer. The
substance was identified by laboratory analysis as PPD using gas chromatography mass spectroscopy.

1.4.2. Chronic Toxicity

The prolonged use or exposure to PPD was reported to result in severe allergic and toxic reactions. Brown et al. (1987) reported two cases of ladies (51 and 62 years old) who were admitted to hospital suffering chronic renal failure. Investigations indicated that they habitually used hair dye with PPD base. Isreal et al. (1934) reported a case of 21 year old lady who worked for five years in a hair dressing department as a dyer. There was a subacute atrophy of the liver possibly as a result of PPD poisoning. Eighteen cases of hair dressers who spent five years in work were found to develop hand dermatitis. It was diagnosed as contact allergy due to PPD (Nethercott et al., 1986).

1.5. Clinical Picture

1.5.1. Acute Toxicity

Percutaneous absorption and ingestion produce severe local and systemic effects. Local reaction include severe dermatitis, urticaria in the eyes, chemosis, lacrimation, exophthamous, phthalmia and even permenant blindness. The systemic action include, asthma, gastritis (regardless of route of entry), rise in blood pressure, transudation into serous cavities, vertigo, tremors, convulsions and coma (Gleason et al., 1963). It was stated in (Martindale, 1952), the following effects result from PPD: local effects which consist of oedema and severe dermatitis and systemic effects that results in vertigo, gastritis, asthenia, deplopia, chemasis, exophthalmos, asthma and exfoliative dermatitis. Goutier (1909) pointed out that a single application of PPD is sufficient to produce a toxic action, skin eruptions, eczema, and urticaria with great burning and itching. Gastrointestinal symptoms such as nausea and nervous symptoms, sleeplessness, dizziness, weakness of legs, epileptiform attack and syncope.

Ingestion of PPD produce two types of toxic effects. The first consist of rapid development of severe oedema of the face, tongue, pharynx as well as laryngeal oedema
with acute respiratory distress which requires emergency tracheostomy. In the latter phase rhabdomyolysis developed followed by acute tubular necrosis (Averbukh, et al., 1989). Acute PPD poisoning shows characteristic face presentation which by no means could be mistaken for other causes. The characteristic chocolate brown colour of urine could be confirmative evidence of PPD poisoning in individual with characteristic face oedema even in absence of laboratory facilities and when history is lacking and in case of emergency (Yagi et al., 1991).

In cases reported the clinical picture may vary due to the amount of PPD ingested and the susceptibility of the individuals. The eighteen cases reported by Yagi et al. (1991) were classified into serious and mild cases. The former were all drowsy and had marked oedema of head and neck, respiratory distress, dysphagia, disability to give history and need tracheostomy. While in the mild cases symptoms were less pronounced and in addition patients give history of vomiting. All the cases showed dysphagia and only one suffered exophthalmos with permanent blindness. The time of onset of symptoms after ingestion of PPD was about 3-6 h. However in the case reported by El-Ansary et al. (1983), toxic effects developed within two hours following ingestion. It started with vomiting and quickly progressed to severe oedema of the face, neck and pharynx. In more severe cases the larynx and upper airways rapidly became affected leading to acute respiratory difficulty and require emergency tracheostomy. The presentation was like that of angioneurotic oedema. Epigastric pain and painful facial oedema with slight dysphagia followed in two hours time. At terminal stages there was severe respiratory failure and trismus. These symptoms were shown in the case reported by Baud et al. (1983). However in the case reported by Suliman et al. (1983) there was immediate numbness and burning of mouth and throat that developed an hour later, followed by swelling of lips, tongue, neck and finally the patient became dyspnoeic and drowsy. Muscle rigor with tenderness initially developed in the lower extremities and subsequently extended to all over the skeletal muscles. This occurs together with the known symptoms of PPD poisoning which include larynx oedema and vomiting (Saito et al., 1990). Lifshits et al. (1993) reported a case of PPD poisoning that suffered from sore throat, cough and anorexia followed by severe dyspnea caused by oedema of
tongue, pharynx and neck. Dyspnea and severe oedema were also shown in case reported by Averbukh *et al* (1989) and Ashraf *et al* (1994). However in the case reported by the latter the patient suffered cardiac death which occurred after admission.

1.5.2. Chronic Toxicity

In most cases of prolonged exposure to PPD, dermatitis have been shown in most cases together with other effects. Brown *et al* (1987) stated the following picture: several small nodular lesions over knuckles and elbows. The patient also showed anorexia, myalgia, lethargy, swelling of the ankles and nodular lesions over elbows. Israel *et al* (1934) reported a case of a dyer working in hair dressing department for five years, the patient developed general weakness, slight abdominal pain and the skin was jaundiced with purpuric haemorrhages over the upper part of the chest, shoulder, and lower abdomen.

1.6. Treatment

There is no specific antidote for PPD poisoning. Cases admitted to hospital usually receive symptomatic treatment. The major early challenge to life is asphyxia followed by respiratory difficulty and the patient appears to be drowsy. Tracheostomy is always the first line of the treatment of symptoms (Yagi *et al*., 1991). In the case reported by Baud *et al* (1983) nasotracheal intubation was found to be responsive, however tracheostomy was found to be successful in about 70% of the cases reported by Yagi *et al* (1991). Renal failure was found to be the second life threatening effect of PPD intoxication and is treated with haemodialysis.

Treatment is symptomatic since there is no specific antidote for PPD intoxication. Symptoms that predominate seems to be histamine-like effects, so therapeutic trial with antihistamine was suggested by Gleason (1963). In cases reported by Yagi *et al* (1991), intensive medical treatment by hydrocortisone and chlorpheniramine meleate was given concomitantly, penicillin cover was given to all patients. This treatment was consider to be life saving, however diazepam was given to patients who developed convulsions. The case reported by Saito *et al* (1990) have been treated by pethedine for
relief of the severe muscle pain with anti-inflammatory, but the patient died as a result of renal failure. In the case reported by Averbukh et al. (1989), inspite of the treatment with adrenaline and massive doses of steroids the patient rapidly developed upper air-ways obstruction which required tracheostomy. Acute renal failure was treated with haemodialysis for three weeks. Full recovery was obtained five weeks following admission. Steroids and dialysis may lead to a favourable result as stated by Suliman et al. (1983) who reported a case of PPD poisoning. The case was treated with dexamethasone and peritoneal dialysis. Urine flow was re-established on the fifth day of admission. The biochemical picture returned to normal after five weeks. The patient gained full recovery and maintained normal renal functions. In one of the cases reported by Brown et al. (1987) treatment with prednisolone resulted in improvement. However the patient died from coliform and bronchopneumonia despite peritoneal dialysis and steroid treatment. The other case was given prednisolone, she remained oliguric and three weeks later developed non-reversible ventricular fibrillation while receiving haemodialysis. In the case reported by Lifshit et al. (1993), despite treatment with antibiotics irreversible ventricular fibrillation developed and the patient died eight hours after admission.

1.7. Experimental Studies

The experimental studies tackling PPD toxicity were very few. They were devoted to determination of lethal doses, absorption, hypersensitivity test, carcenogencity and mutagancy of PPD. The studies focused on PPD as a constituent of hair dye formulations, material dyed with PPD and in the working atmosphere. Areas like PPD ingestion for suicidal, homicidal and accidental purposes in pure form did not gain much concern. This may be due to the infrequency of cases reported in the developed countries. Experiments dealing with carcenogencity of PPD did not reveal positive results. The chairman of the skin and cancer hospital, the Temple University (USA), has stated that absence of skin cancer in people known to be exposed to carcinogenic amine led to the conclusion that there is no evidence for skin cancer associated with the use of hair dye. The conclusion is further supported by experiments conducted in animals and
incidental observations, (Burnett et al 1977).

1.7.1 Lethal Dose

The oral LD$_{50}$ of PPD was studied by Spector (1956), he found that the LD$_{50}$ was 250 mg/Kg bw in rabbits and 100 mg/Kg bw in cats. However the subcutaneous LD$_{50}$ was found to be 170 mg/Kg bw in rats, 200 mg/Kg bw in rabbits and 100 mg/Kg bw in dogs. Burnett et al (1977) studied the LD$_{50}$ in rats and found that the intraperitoneal LD$_{50}$ of PPD in dimethysulphoxide was 37 mg/Kg bw. The oral LD$_{50}$ of PPD in oil-in-water emulsion in rats was found to be 80 mg/Kg bw. Intraperitoneal administration of PPD produced a characteristic oedema of head and neck of rabbits and cats (Tainter et al., 1924). The same effects were seen in guinea-pigs and rats. Studies of Mascre and Jasmin (1974; 1975) demonstrated that subcutaneous administration of 3 mg of PPD hydrochloride induced skeletal muscle lesions, rhabdomyolysis with infiltration of myophages, necrosis with calcification, accumulation of neutral lipids and dilation of sacroplasmic reticulum.

1.7.2 Absorption

Absorption of PPD through the skin, metabolism and excretion of PPD have received little attention. In experimental studies carried by Manfred et al (1968) the absorption of PPD through the skin was calculated from the concentration determined in blood or the amount excreted in urine. When PPD (1.5g in 1:1 isopropanol in water) was applied to the skin of the dog, it was calculated that 16mg were absorbed in 3 hours via the skin (area of 500 cm$^3$) when the animal have free access to air. The amount absorbed increased when covering the applied PPD with aluminum foil immediately after application. In another experiment performed by Mathur et al (1990) the biochemical and histopathological changes following dermal exposure to PPD were studied. Guinea-pigs were painted with 1% solution (w/v) of PPD in 25% ethanol over an area of 2x2 cm clipped free of fur for 1, 3, 5 and 7 days. It was observed that PPD was absorbed through the skin into the serum and excreted in the urine. The study indicated that PPD exposure resulted in increased level of histamine contents of skin.
The latter is a sign of hypersensitivity reaction. The activity of enzymes GOT, GPT, Tyrosinase and γ-Glutamyltransferase (GGT) increased significantly in serum and skin. Degenerative changes were observed in the liver. The dermis cells showed hyperkeratosis together with infiltration. Biochemical and histopathological changes in skin and serum correlated with the duration of exposure to PPD. Hyung et al (1987) carried out experiments to determine the skin absorption of PPD from a variety of patch test systems. [14C] PPD (1%) was placed in each system at concentration normalized to equal surface area (2mg/mm^2). Skin absorption was determined in guinea-pig by urinary excretion of [14C]. The highest percentage absorbed was found to be 53.4% of the applied dose.

1.7.3. Toxicity

Averbukh et al (1989), after reporting a case of PPD toxicity has performed animal experiment to provide more insight into the quantitative aspects of the toxicity of PPD. Two groups, 15 mice each, were given PPD 70 mg/Kg bw and 35 mg/Kg bw respectively. They were then sub-grouped and sacrificed 24, 72, and 120 h after ingestion. The enzymes CPK and aldolase showed increased activity after 24 h in both groups. Serum CPK activity decreased considerably after 72 h, but still remained significantly elevated in both experimental groups compared to control. After 120 h all values converged to normal. In animals sacrificed after 24 h there was acute rhabdomyolysis with fresh segmental necrosis of myofibres characterized by pyknosis of subsarcolemmal nuclei and fragmentation of the sacroplasm. At 72 h these changes appeared together with necrosis, proliferation of the satellite cell and infiltration of macrophages with evidence of phagocytosis. At 120 h there was regeneration and an increase in the number of nuclei. Raws of central nuclei were apparent in regenerating myofibres. No abnormality was observed in histological examination of kidneys and liver sections. In a study carried out by Yabe (1992) to clarify the mechanism of PPD related occurrence of rhabdomyolysis, the pharmacological effect of PPD, on the function of contractile proteins and the sacroplasmic reticulum have been investigated in a single muscle fibre of the rat using the skinned fibre method. The following results
were obtained:-

1- A positive contraction of the skinned fibre by PPD was noticed, suggesting that contraction of the muscle by PPD is caused by the release of Ca$^{2+}$ from sarcoplasmic reticulum (SR).

2- PPD inhibited the Ca$^{2+}$ uptake by SR in a concentration dependant manner and greatly accelerated Ca$^{2+}$ leakage from SR.

3- The calcium-induced calcium release (CICR) was accelerated in areas with low Ca$^{2+}$ concentration. Thus it has been speculated that PPD can bring about rhabdomyolysis by promoting CICR and leakage of Ca$^{2+}$ from the SR. This was followed by an increase in the Ca$^{2+}$ concentration and by consequent changes that developed in the muscle such as continuous contraction and irreversible changes in the muscle structure and/or hypermetabolic changes.

Experimental studies performed by Jain et al (1979), conducted in rats and rabbits revealed that the hair dye possesses cataractogenic effect on lenses, and they concluded that the hair dye PPD is potentially toxic to human lenses.