From this study it was found that the commercial hair dye which is available in the local markets possesses the same chemical and physical characteristics of authentic PPD. This was proved by TLC, U/V, IR, MP and X-ray fluorescence. TLC, U/V and I.R showed the same Rf, and U/V. and IR. Spectrum of both samples (authentic and commercial) of PPD. X-ray fluorescence showed PPD commercial sample spectrum free of metal impurities that may interfere with the toxicity of PPD. The melting point of PPD (authentic and commercial) was within the range (145 - 147°C) of PPD stated in Merck Index (1983). These results were compatible with the specification of manufactured PPD which has a purity of 99.2% in U.S.A, 99% in UK and 99.5% in Japan (IRAC monograph, 1977).

Toxicology experiments were carried out to evaluate the hair dye (Commercial PPD) toxicity in a way to estimate the hazards of this compound to humans since it is known that toxic effects in humans are usually in the same range as those of experimental animals. However humans are generally more vulnerable than experimental animals by a factor of ten (Curtis, 1986). Variation in toxic effects between authentic and commercial PPD were negligible. This indicate that hair dye (commercial PPD) possesses the same toxicological properties as those of authentic PPD. However variations if present may be due to partial oxidation of the material on exposure to light as a result of improper storage.

Different species were used in this study to provide more information about toxicity of this compound as recommended by Curtis (1986). Symptom were seen to be similar in both species, chicks and rats, but the time of onset was shorter in rats. Fatality occurred at lower doses in rats (35 mg/kg bw of PPD) compared to lethal doses noticed in chicks (140, 105 mg/kg bw of PPD) when administered subcutaneously. With sublethal doses of PPD, deviation from normal values in biochemical and haematological parameters was wider in rats than in chicks. For example enzymes GOT
activities were shooting very high in rats after administration of PPD, while in chicks it was found that elevation of the activities of these enzymes was not that high compared with the values of control in each species. Rats were susceptible to PPD toxicity and further susceptibility might be expected in humans. Species variation may be due to different rates and patterns of biotransformation of PPD. Rate of elimination of PPD from the body in different species may also contribute to species variation (Curtis, 1986).

The administration of a toxic agent via variable routes may give an idea about the biotransformation and excretion. In the present study PPD was introduced through subcutaneous, intramuscular and oral routes. Toxicological changes following exposure were similar, but differ in severity. Subcutaneous dose of PPD in chicks (140 - 105 mg/kg bw) and in rats (35 mg/kg bw) caused fatality in both species, however the same dose did not cause death in the two species when administered orally. This may be due to the effect of hydrochloric acid which form a more polar less absorbable compound, or due to intestinal flora found in the gastrointestinal tract. The action of the latter factor on PPD may alter the parent compound to less toxic material. Curtis (1986) has stated that snakes venom is not toxic when administered orally because it is broken down by digestive enzymes in the gastrointestinal tract. Fatality that came as a result of PPD toxicity via oral route have been reported (Saito et al., 1990 and Ashraf, 1994). The administration of subcutaneous and intramuscular single dose of PPD in chicks (70 and 35 mg/kg bw) and in rats (17.5 mg/kg bw) showed toxic effects without fatal consequences up to 24 h before slaughtering. Variation in systemic toxicity of PPD between subcutaneous and intramuscular route of administration was not that great. Absorption via subcutaneous and intramuscular routes is influenced by blood supply to the site of injection. However subcutaneous injection results in a rather faster absorption than intramuscular injection but the difference is not great (Curtis, 1986). Spector (1956) have stated that the subcutaneous LD$_{50}$ of PPD is 170 mg/kg bw in rats. While the oral LD$_{50}$ of PPD in water emulsion in rats is 80 mg/kg (Burnett et al., 1977). This confirm that HCl of stomach render PPD less absorbable and the emulsion form
enhance absorption. Systemic toxicity of PPD with different doses indicate dose
dependance of this toxic agent in both species. At higher doses of PPD there was broad
deviation from the normal values in biochemical and haematological parameters
compared to lower doses of PPD administered via the same route in the same species,
because the concentration of a toxic agent influence its rate of absorption. At high
concentration of a toxic agent rapid absorption occurs, giving rise to high concentration
at the target organ and will result in high toxic effect. Curtis (1986) has stated that the
production of a response and the degree of the response are related to the concentration
of the toxic agent at the target site. Toxic effects of PPD are produced when the toxic
agent or its metabolites reach the appropriate site in the body at high concentration and
for a length of time sufficient to produce toxic manifestations. This require the
absorption and distribution of the toxin from its entry point to a distant site at which
deleterious effects occur.

Pathological investigations carried out in chicks that received single dose of PPD
(35, 70, 105 and 140 mg/kg bw) and rats that received single dose of PPD (17.5, 35 and
70 mg/kg bw) showed clear damage to liver, kidney, heart and skeletal muscles. Curtis
(1986) has stated that the rate of distribution of a toxin to the tissues of each organ is
determined by the blood flow through the organ and the ease with which the chemical
cross the capillary bed and penetrate the cells of the particular tissue. Liver damage
associated with PPD have been noticed in cases reported by Israel et al (1934) and
Mathur et al (1990). However in experiments in mice treated with PPD, histopathological changes were observed in skeletal muscles and not in liver or kidneys
(Averbukh et al., 1989). Saito et al (1990) have shown that renal and skeletal lesions
were usually associated with PPD toxicity.

Hepatotoxicity induced by PPD is not surprising since liver injury induced by
chemicals is well authenticated in the literature. Chemicals induce liver injury by
accumulation of lipids, and appearance of degenerative processes leading to death of
cells (necrosis). Carbon tetrachloride (CCl4) which is considered as a reference for
chemicals that induce liver injury was found to affect cellular membranes (Gabriel et al.
The membrane of the mitochondria is also affected by CCl₄ as shown by Christie et al. (1954). In the present work, cell necrosis and fatty changes were noticed in studied tissues. The mechanism by which CCl₄ affect liver cells causing lesions was well studied. Slater (1966) proposed that homolytic cleavage of CCl₄ which takes place in the endoplasmic reticulum will result in production of free radicals, which could then react with neighbouring lipid-rich materials causing alteration in structures and functions. Recknagel and Glende (1973) has stated that lipid peroxidation theory plays a major role in liver injury caused by CCl₄. The pre-administration of antioxidant in vivo did prevent the appearance of fatty liver and necrosis induced by CCl₄. This indicates the role of lipid peroxidation in liver injury (Alper et al., 1968). Lipid peroxidation and free radical formation were found to be associated with exposure to PPD. Mathur et al. (1990) came to the conclusion that dermal exposure to PPD produced fatty changes in liver of guinea-pigs. The increase in lipid peroxidation and the increase in free radical formation is responsible for the histopathological changes which demonstrated tissue damage. Exposure to PPD had been associated with lipid peroxidation and cytotoxicity as a result of autoxidation (Picardo et al., 1992). These studies indicated that PPD was biotransformed in vivo since free radicals and lipid peroxidation were found to be associated with PPD exposure. The resultant metabolites of PPD biotransformation may be responsible for the necrotic effect produced.

Renal lesions associated with PPD intoxication received much attention because most of the clinical investigators suggested renal dysfunction (Suliman et al., 1983, Brown et al., 1987; Saito et al., 1990). Histopathological investigations when performed in some cases of PPD toxicity, renal lesions were obvious. The kidneys were particularly vulnerable to effects of noxious agents because of their high perfusion rate. Renal damage induced by chemicals was well known. Acute administration of nephrotoxic doses of chloroform (CHCl₃) induced fatty degradation, tubular cast and/or marked necrosis of the proximal tubular epithelium (Rush et al., 1984). The nephrotoxicity of CHCl₃ may be due to chloroform, or its metabolites produced by liver or kidney (Kluwe, 1981). Rush et al. (1983) have confirmed the biotransformation of
CHCl₃ by the enzyme cytochrome P-450 in rabbits. Strik (1968) has come to the conclusion that CCl₄ metabolites were responsible for inducing kidney damage. However renal damage may result from external factors such as decreased blood pressure or volume (Jerry et al., 1986).

The effect of PPD in the skeletal muscles was noticed in cases reported by Averbukh et al. (1989) and Saito et al. (1990). Cardiac muscles in the present study showed clear damage while in previous studies it was not reported. It is known that the chemical effect on cardiac muscles and the cardiotoxicity involve an irreversible interaction between the chemical or its metabolites with a functional or structural molecules of vital significance. It was found that the protective mechanism against free radical in the heart are much lower than that existing in other organs. This makes the heart specially susceptible to the effect of chemicals (Tibor et al., 1986).

The damage of the liver, kidney, heart and skeletal muscles, was reflected in the levels of the biochemical and haematological parameters. There was a significant increase in the activities of the following enzymes GOT, GPT, LDH, ALP, CPK and aldolase, in addition to the increase in the concentration of glucose, uric acid, potassium and calcium. There was also a significant decrease in total serum proteins, cholesterol and magnesium. These changes were noticed in dosed chicks. The administration of PPD to rats revealed the following changes: significant increase in GOT, GPT, ALP, creatinine, urea, cholesterol, potassium and calcium and a significant decrease in total proteins, glucose and magnesium. Changes in biochemical parameters in the present study indicate hepatorenal toxicity in addition to cardiac and skeletal muscles lesions. Since PPD produces damage to liver, kidney, heart and skeletal muscles, this will lead to leakage of enzymes contained in the cells of these tissues to the blood and this may be the reason for the high activities in plasma. These findings are in agreement with the findings of Baud et al. (1983), Suliman et al. (1983), Averbukh et al. (1989) and Satio et al. (1990). Renal toxicity of PPD predominate since most of the cases reported showed renal failure which became the major cause of death. The plasma activity of GPT, GOT, LDH and aldolase were found to increase following CCl₄ hepatotoxicity.
Increase in GOT with a decrease in total proteins were observed with hepatorenal toxicity in chicks fed Jatropha (Elbadwi et al., 1992). Serum proteins are synthesized mainly in the liver. Increase in plasma concentration of uric acid may be due to renal damage as suggested by Ayed et al (1991) and Ibrahim et al (1992). Hyde (1983) stated that elevated levels of uric acid, creatinine and urea are associated with renal impairment. Rush et al (1984) observed similar findings and also observed an increase in blood urea, proteinuria in CHCl₃ nephrotoxicity. Proteinuria may contribute to the significant decrease in plasma total proteins noticed in the present study. Hyde (1983) stated that glomerular damage may result in insufficient reabsorption of proteins which may lead to loss of proteins in urine. PPD was found to cause an increase in the serum level of K (Suliman et al., 1983). Altered membrane permeability and cations (Ca²⁺, K⁺ and Mg²⁺) transport has been reported in nephrotoxicity of aminoglycosides (Hume et al., 1982; Kaloyanies 1984). Hyperkalemia is a common finding in renal failure due to the release of intracellular potassium stored in association with cell injury and death. Hyperkalemia resulting from renal failure is most likely to occur if renal failure is acute. Extensive cellular necrosis may cause increase in plasma level of K since most of the K is intracellularly located (Delmar, 1980). PPD toxicity was found to increase Ca level (Satio et al., 1990). Similar increase was observed in this study. Mogens (1980) suggested that hypercalcemia is a secondary rather than a primary manifestation of renal insufficiency in ponies. It was found that bilateral nephrectomy increased the level of plasma calcium indicating the role of the kidney in calcium homeostasis. The decrease of total proteins observed in the present study was not reported in previous studies in PPD toxicity. However it was noticed in previous studies that chemicals have some effects in protein synthesis (Smucker et al., 1961). Dianzani (1979) stated that CCl₄ may interfere or inhibits protein synthesis in the liver. This may be due to the effect of CCl₄ in the endoplasmic reticulum (Recknagel and Lumbardi, 1961). Other chemicals can affect proteins synthesis due to their effect on m-RNA. Example of such chemicals is dimethylnitrosamine (Mizarchi et al., 1962). In the present study chicks treated with PPD showed significant decrease in
PPD showed significant decrease in the plasma level of cholesterol, while in rats it produced an opposite effect. The decreased plasma level of cholesterol observed in chicks may be attributed to liver damage, which is known to be associated with fat accumulation in the liver due to its inability to form and secrete lipoproteins and thus low plasma cholesterol. However the increased level of cholesterol that was seen in rats may be attributed to mobilization of fats from adipose tissue to compensate for the low level that occur as result of liver damage. The ultimate cause of fatty liver and subsequent hypertriglyceridemia is presumably mobilization of free fatty acids derived from adipose tissues (Jone et al., 1965). Hyde (1983) stated that low cholesterol level is usually associated with hepatocellular damage. Recknagel and Ghoshal (1966) suggested that the free radical that arise from homolytic cleavage of CCl₄ in rats attacks the methylene bridge of unsaturated fatty acids chain of microsomal lipids, resulting in morphologic alterations of endoplasmic reticulum. This will decrease protein synthesis and also the capacity of the liver to form and secrete lipoproteins. Block of the secretion of hepatic triglycerides into plasma in damaged liver, induced in rats by CCI₄, was suggested by Lumbardi (1966) and Hoyumba et al (1975). The increase in the level of cholesterol is associated with hepatic lipidosis and obstructive liver diseases (Jack, 1980).

The increased level of glucose that was seen in chicks treated with PPD may be attributed to liver damage, which render the hepatic cells unable to metabolize glucose and this result in high blood glucose level. Varley (1975) has suggested that, severe liver disease reduces the rate of glycogen formation and synthesis of glucose from non-carbohydrates sources (Gluconeogenesis). The same effect may occur in rats and there is high blood glucose level, but renal damage may contribute to loss of glucose in urine, in addition to insufficiency of liver to compensate for this loss. The net result of this interaction is low blood glucose level in rats. Varley (1975) has stated that due to impaired reabsorption of glucose in renal tubules, appreciable amount of glucose escapes in urine. Glomerular damage may result in insufficient reabsorption of glucose, which may lead to loss of glucose in urine (Rush et al., 1984). In chronic toxicity
experiment in the rats blood glucose level was high, this may be due to the low level of insulin as a result of the effect PPD on pancreatic cells (islet cells). However histopathology of the pancreas was not carried out in this study. Insulin deficiency is associated with high level of blood glucose, since it is known that insulin facilitate glucose entry into peripheral tissues and also influence the metabolism of glucose by the liver (Jiro, 1980).

The plasma level of Mg was found to decrease significantly in both chicks and rats treated with PPD in the present study. Magnesium deficiency is frequently observed in cirrhosis of liver (Frink 1978; Hamed et al., 1978 and Schroeder et al., 1969). There is no previous study linking Mg with PPD toxicity.

Damage of cardiac and skeletal muscles noted in this study was further confirmed by the increase in the activity of LDH, CPK and aldolase. Necrosis of the myofibres is an example of a process by which serum activities of intracellular enzymes are elevated. Heart and skeletal muscles usually contain sufficient amount of CPK that can alter serum activity in organ specific disorder (John, 1980). LDH and aldolase were known to be associated with increased permeability or dysfunction of muscle tissues (Hyde 1983). PPD toxicity have been linked with rhabdomyolysis and skeletal muscle lesions as well as with elevation of the level of associated enzymes (Averbukh et al., 1989 and Sato et al., 1990). The effect of PPD on the skeletal muscles was studied by Yabe (1992) and he suggested that rhabdomyolysis results from the action of PPD on sarcoplasmic reticulum and leakage of Ca$^{+2}$ ions which give rise to irreversible changes in the muscle structure and/or hypermetabolic changes.

Biotransformation of PPD may explain structural and functional changes noticed in liver, kidney, heart and skeletal muscles however PPD itself may contribute to these lesions. PPD metabolites or intermediates toxicity are more responsible than that of parent PPD, since PPD as such was not detected in these organs in this study. Strong binding of PPD with the tissues proteins may occur which may make detections of PPD difficult. For this reason we tried to precipitate proteins so as to detect the dye, but we failed to precipitate the pores due to the strong linkage between the dye and the
Biotransformation of PPD may provide reasonable explanation for not detecting the dye in chicks and rats organs. This is strengthened further by the lack of direct effect of PPD on isolated tissue preparation as it will be discussed later.

Toxic effect of PPD was monitored during a course of 120 h after a single dose of PPD given to chicks. Histopathological findings seen in liver, kidney, heart and skeletal muscle at 24, 72 and 120 h were similar indicating no more change with time. It also indicates that the damage produced in these organs is irreversible. However biochemical parameters revealed signs of slight recovery but still changes were significant compared to the normal values (control). The enzymes GOT, GPT, ALP adolase activities increased to maximum values at 72 h. At 120 h values tended to decrease while still significantly higher than the control. Serum Total proteins and cholesterol showed maximum decrease at 24 h. Uric acid and Ca concentrations were higher at 120 hours. These findings indicate that hepatorenal toxicity caused by a single dose of PPD will continue at least up to 120 h without complete recovery. The highest values of PCV and MCHC were noticed at 72 h.

When considering the effect of time in rats it was found that incomplete recovery was noticed up to 120 h. Increase in the activities of GOT, GPT and ALP was higher at 72 h. Total proteins and glucose were lower at 24 h. Urea, creatinine and cholesterol were higher at 24 h. This indicates that hepatorenal toxicity of PPD was higher at 24 h in rats and at 72 h in chicks. Similar results were reported in the experiment carried out in mice. The maximum toxicity was observed at 72 h (Averbukh et al., 1989). This reaffirm susceptibility of rats to PPD toxicity more than chicks. Complete recovery was not attained up to 120 h in both species. This is not surprising, since the toxic response is dependant on the rate at which toxic metabolites are produced and detoxified. Animals are known to possess the ability to biotransform and to excrete toxins. Biotransformation convert readily absorbed (lipophilic) compound into more polar and less lipid soluble compound. This enhances water solubility, reduces distribution, enhances detoxification and promotes excretion. Curtis (1986) has stated that the action of drug metabolites are actively terminated by further biotransformation and/or by
excretion of active metabolites. When the rate of absorption exceeds the rate of elimination, the toxic compound may accumulate to critical concentration in the body and toxic effects are then observed. The accumulation of toxic agent may serve to prolong the action of the toxic agent at the reactive site. Toxic effect usually disappears when the concentration of the toxic agent in the tissue is decreased by excretion from the body. Reversibility of the toxic action may occur when the concentration of the toxic metabolites does not exceed the critical concentration. The complete recovery from PPD should be influenced by factors like absorption, distribution, biotransformation and excretion. In addition to the fact that the liver have the ability to regenerate its cells.

In one part of this study we tried to create chronic PPD toxicity in chicks and rats. Chicks and rats were injected subcutaneously with a single dose of PPD (45, 17 mg/kg bw respectively) per week for six weeks. There were toxic effects without fatal consequences during the stated period in both species. Lesions were observed in liver, kidneys, heart and muscles. These lesions resulted in biochemical and haematological changes which include increase in the level of GOT, GPT, ALP, in both species. There was an increase in activities of LDH, CPK and aldolase in chicks and creatinine in rats. There was a significant increase in urea, uric acid, glucose and cholesterol with significant decrease in total proteins. These changes resulted from hepatorenal toxicity of PPD administered over a long period of time of chronic toxicity of PPD.

In another experiment the pharmacological effects of PPD was tested in vitro using, rats, rabbits, guinea pigs, frog and cats. PPD did not affect striated muscles, since the addition of this substance to the frog rectus abdominus muscles preparation did not produced antagonistic or agonistic effect. However increasing the dose produced irreversible reduction or total loss in the sensitivity of the tissue to carbachol. This effect was probably attributed to necrotic action of PPD (Yabe, 1992). The electrically stimulated striated muscles (diaphragm) twitches were not affected by the addition of PPD. However at higher doses the tissue did not respond to the electrical impulses. This may be due to the necrotic action of PPD on tissues, nerves or both.
PPD in small doses did not affect isolated rat uterus (non-contracting), although it is rich in receptors that mediate contraction like muscarinic, and 5-hydroxytryptamine receptor, in addition to β-adrenoceptor that mediate relaxation (Kitchen, 1984). However the addition of larger doses of PPD produced irreversible dose-dependent reduction or total loss in the sensitivity of the tissue to carbachol. This can also be attributed to the necrotic effect.

Rat fundus strip did not respond to the addition of PPD. The sensitivity of the tissue to 5-hydroxytryptamine decreased and totally lost after exposure to higher doses of PPD. Necrotic effect of PPD on the tissue may be the major cause that abolished the sensitivity of tissues to the drug. Similar experiments have been conducted on skinned muscles and indicated that PPD may lead to leakage of Ca$^{2+}$ from sarcoplasmic reticulum and consequently changes developed in the muscle such as continuous contraction and finally give rise to irreversible changes in the muscle structure (Yabe, 1992).

Rat aortic strip was not affected by the addition of PPD, although it possesses α and β-adrenoceptors as well as muscarinic receptor (Kitchen et al., 1984). This confirmed the insensitivity of adrenoceptor and cholinoreceptors to PPD. However the sensitivity of this tissue was not affected by higher doses of PPD. Also the blood pressure of anaesthetized cat did not change by intravenous injection of PPD.

Rat ascending colon did not respond initially to exposure to PPD, but the sensitivity of the tissue to the drug was reduced and totally lost after addition of higher doses of PPD. The activity of ascending colon is known to be mediated via prostaglandins (PGs) (Vane, 1971). This may indicate that PPD did not have PG-like activity, nor having the ability to alter the synthesis of endogenous PG. Similarly the lack of effect of PPD on the colon may indicate that the dye does not affect the autonomic innervation of the preparation.

PPD produced a significant positive inotropic (increase in force) and chronotropic (increase in rate) effect of the isolated perfused rabbit heart. This effect cannot be attributed to β-adrenoceptor stimulation since it was not blocked by pre-addition of
propranolol. This confirmed the insensitivity of adenoceptor of the heart to PPD. The effect produced by PPD on the heart was blocked by pre-addition of chlorpheniramine (anti-histamine). This may be due to the stimulation of \( H_1 \) and \( H_2 \)-receptor of the heart which has inotropic and chronotropic effect (Douglas 1985). PPD may interact with \( H \)-receptors to produce the same effect of histamine and is considered a histamine-like substance. Alternatively PPD may induce histamine release from the mask cells and as result considered as histamine releaser.

Previous studies have shown that cardiac muscles continuously produce measurable quantities of histamine (Anrep, 1936) however Giotti et al (1966) have considered the isolated heart a suitable test for the study of histamine release. In the study performed by Monger et al (1952) it has been found that the amount of histamine released by chemical releaser is the same as that released by antigen. Tibor et al (1986) have stated that cardiovascular toxicity is represented by the immune system-mediated effects. In which the hypersensitivity reaction that occurred may be due to the fact that the compound acts as hapten which bind to an endogenous macromolecule or the compound may act directly on the immune system. The protein bound hapten binds to the antibody immunoglobulin (IgE) on mask cells and elicit the release of histamine.

Incubation of PPD with lung tissues produced a clear contraction of guinea-pig ileum, when the supernatant was added to the tissue although the dye alone did not affect the tissue. This effect was mediated via \( H_1 \)-receptor effect, since it was blocked by pre-addition of chlorpheniramine. The experiment showed the histamine releasing property of PPD. The contraction of the ileum was not seen on addition of a blank incubate (incubation in absence of lung tissue). Guinea-pig lung tissues are known for its highest contents of mast cells, and usually used to study the histamine released by different factors (Schild, 1959; Kitchen, 1984). Guinea-pig ileum is highly responsive to histamine since it is rich in \( H \)-receptors (Kitchen 1984). Monger et al (1952) have studied the effect of the histamine release of 48/80 compound by incubating the compound with guinea-pig lung tissues and the histamine released was assayed.
biologically using guinea-pig ileum. Brezezinka - Blazezyk * et al (1987) found that guinea-pig mesentric cells and pulmonary tissues were among the most susceptible tissues to histamine releasing factors.

Exposure to some chemicals and drugs have been associated with allergic reactions in both humans and animals, so the immune system have been implicated as a target organ when studying toxicity of such compounds (Jack * et al., 1986). Challenge with antigen or the chemical compound 48/80 evoked mast cells degranulation and histamine release (Raud, 1989). The latter is known for its action on mast cells and histamine release (Aldenborg 1989). It is usually used for studying anaphyalactic reactions and as a reference compound in studying other histamine release (Arrigo-Reina, 1987 and Saito * et al., 1990). PPD may be included with the group of compounds that possess histamine releasing property.

The mechanism by which PPD release histamine from the mast cells is not known. However the mechanism varies with different chemical release or releasing factors. It may involve impairment of the cellular event linked to exocytosis (Hazama * et al., 1992). Others may disturb the cell membrane leading to cell lysis (Lou * et al., 1996). The release of histamine from the mast cells have been associated with opening of certain calcium channel (voltage-sensitive), leading to elevated intracellular Ca$^{+2}$ level which in turn activate mast cell secretions (Eleno * et al., 1990). Saito * et al (1990) suggested that Braxin A, releases histamine from the mast cells with cytotoxic action on cytoplasmic membranes.

The histamine releasing property of PPD may provide a possible explanation for the increase in the rate and force of rabbit isolated heart, since histamine is known to have direct action on the heart. It increases force and rate by promoting calcium influx and hastening diastolic depolarization in the sinoartial (SA) node. It also act directly to slow the artioventricular (A V) conduction to increase automaticity and elicit diverse arrhythmia (Douglas, 1985). All of these effects were largely attributed to H$_2$-receptor except that of A V conduction to H$_1$-receptor stimulation (Douglas, 1985). But the increase in contratility of rabbit isolated heart was blocked by chlorpheniramine.
which acts mainly on $H_1$-receptor. This might be due to species variation or loss of selectivity in larger doses. PPD being a histamine releaser is without any effect on autonomic nervous system as well as the neuromuscular junction.

The immune system appears to be the major target of PPD toxicity as its action involves release of histamine. Interaction between releasing factors and receptors on mast cells triggers the release of histamine contained in the mast cells resulting in hypersensitivity and allergic reaction. PPD toxicity have been associated with hypersensitivity and allergic reaction. In the study performed by Mathur et al. (1990) it was noticed that the increase in histamine level, as a result of PPD exposure, is attributed to increased mast cell permeability. It is a sign of hypersensitivity reaction. Picard et al. (1992) has stated that PPD autoxidation is considered to be an essential part of the pre-immunological phase in the induction of allergic contact dermatitis. However it had been found by Rajaka et al. (1970) that benzoquinone formation plays an important role in the allergic reaction of PPD. Ng-SK (1990) considered PPD as one of the chemicals that produce different type of reactions such as allergic contact dermatitis, and immunologic contact urticaria. Beside that other types of contact dermatitis were observed. It was found that challenging with PPD produced a positive reaction in humans (Matsukubo, 1990 and Zhao et al., 1991).

Considering the histamine releasing property of PPD and the different types of allergic reactions produced will explain the toxic effects of PPD. For example the characteristic feature of PPD toxicity is oedema of neck and head. Oedema formation is implicated with signs of type I allergic reaction, that results in immediate hypersensitivity mediated by haemocytotropic antibodies IgE (Jack, 1986). However oedema of head, neck and pharynx had been clear in the present study in animals that received higher doses of PPD in both chicks and rats within the first 2 hours after administration. Oedema formation have been associated with most of the cases reported as PPD toxicity (Averburkh et al., 1989; Yagi et al., 1991; Lifshit et al., 1993). This effect may be due to the histamine action on the vascular system. Capillary dilatation is the characteristic action of histamine on the vascular system. It involves both $H_1$ and
H₂-receptors which lead to fall in resistance upstream (increased blood flow), combined with the action of histamine on large veins which will lead to contraction of venules (Bowman and Rand, 1980). Accumulation of blood in capillaries increase capillaries permeability and this will lead to fluid and proteins loss from the circulation into surrounding tissues leading to formation of oedema (Douglas 1985). The result of this is a decrease in the volume of circulating blood. The reduction of the blood volume in this study have been observed in animals that received large doses of PPD, especially rats. Haemoconcentration was a clear sign compared to undosed animals (control).

In the present study, haematological investigations showed that there was a significant increase in Hb and PCV values and may be attributed to the escape of plasma from circulation to the surrounding tissues. This effect was noticed in chicks treated with PPD and rats that received lethal doses of PPD. In addition chicks showed a significant decrease in MCHC value while rats showed a significant decrease in RBCs and MCHC with a significant increase in MCH and MCV values. These haematological changes indicate that anaemia may occur as a result of exposure to PPD. The possible cause for anaemia is the haemolytic effect of PPD on RBC. Anaemia was obvious in rats that received sublethal doses of PPD however in chronic toxicity experiments, both species showed haematological changes indicating anaemia. These changes include a decrease in the values of Hb, PCV, RBC and MCHC with an increase in the values of MCH and MCV. Roger et al (1986) have stated that anaemia can arise if for any reason the rate of red cells destruction in peripheral blood exceeds the normal rate of production in bone marrow. Some chemical and/or its metabolites are recognised as having direct haemolytic effect on red cells. However damage may occur as a result of impaired oxygen transport. The effect of chemicals may extend to bone marrow leading to inadequate production of red cells and other elements. The increase in MCV and MCH have been associated with macrocytic anaemia, while the decrease in MCHC values indicate anaemia and iron deficiency (Hyde 1983). Anaemia have been noticed in the cases reported by Brown et al (1987). These cases resulted from chronic exposure to PPD. However in acute toxicity of PPD haematological changes were not
TWBC count was found to increase in rats that received different doses of PPD. This may be due to the action of PPD in the immune system, which triggers massive production of immunocompetent cells. Jack et al. (1986) have stated that exposure to chemical have been associated with quantitative changes in peripheral Leukocytes and differentials counts. An excess production of granulocytes occur after administration of some drugs like epinephrine.

The effect of diminished blood volume induced by histamine will reduce venous return and greatly lower cardiac output (Douglas, 1985). The cardiac output may fall percipitously to a point at which cerebral circulation is not sufficient to maintain consiousness (Bowman and Rand 1980). Yagi et al. (1992) stated that severe PPD toxicity has been associated with unconsciousness. Douglas (1985) produced another explanation for the reduction of blood volume. They attributed that to the histamine action on post capillary venules. Histamine may cause the endothelial cells to contract their boundries and thus expose basement membranes which will be freely permeable to plasma proteins and fluids. The gap between the endothelial cells may also permit passage of fluids and proteins that become trapped between the endothdial cells and the basement membrane. The leakage of plasma proteins with the loss of fluids may contribute to the significant decrease in plasma level of total proteins in dosed animals and provide reasonable cause for oedema formation. The glomeruli are the primary site for the action of several chemicals. Glomeruli are susceptible to immunologic injury following toxic effect (Jack et al., 1986). The action is mediated by immunoglobulin G (IgG) through a mechanism involving the generation of antigen-antibody complex that subsequently fix complement. This complex became deposited in the vascular endothelium where destructive inflammatory response occurs and results in glomerulonephritis (Jack et al., 1986).

Histopathological changes in the present study showed marked necrosis in the kidney of dosed animals and immune toxicity may contributes to this effect. Cytotoxic reactions which are mediated by IgG and IgM antibodies possess the ability to fix
target organs were kidneys and circulatory system resulting in glomerulonephritis, haemolytic anaemia and haemorrhage (Jack et al., 1980). This may explain the decrease in RBC count observed in this study. Destruction of blood cells may involve an allergic mechanism (autoimmune haemolytic anaemia) after sensitization by chemicals as exemplified by action of acetonitril (Roger, 1986). Bowman and Rand (1980) has stated that smooth muscles in the walls of hepatic veins in dogs contract in response to histamine. The portal vein and mesentric veins, as a result, become engorged with blood. Congestion of liver and small intestine that appeared in post-mortum of animals in the present study may be attributed to this effect. Respiratory distress and dyspnea have been shown in animal that received large doses of PPD. In several previous studies both effects were noticed in severe PPD toxicity (Bourguia et al., 1988; Yagi et al., 1992 and Lifshit et al., 1993). Martindale (1952) and Gleason (1963) included asthma as one of the symptoms of PPD toxicity. In the study performed by Manning et al (1987) bronchoconstriction induced by histamine was obvious. Douglas (1985) has stated that a large dose of histamine caused profound bronchospasm and dyspnea.

It can be concluded from this study that:

1. Hair dye in crystal form, which is available in the market is PPD and the administration of commercially available PPD to animals produced similar toxic effects as that of authentic PPD.

2. PPD produced hepatorenal toxicity regardless of the route of administration. Liver and kidney are the main target organs to PPD toxicity, however heart and skeletal muscle may also be included.

3. Most of PPD toxicity symptoms are due to the release of histamine. The immune system can be considered as a target organ in PPD toxicity, however the link between PPD histamine release and immune system worth further investigations.


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الملخص الإطروحة

اجريت عدة تجارب لمعالجة الاثر السام لمادة الPPD (صيغة الشعر) على اثنين من فصائل حيوانات التجارب. عل سبب المقارنة بأعضا الحيوانات المادة بنسب متفاوتة بعدة طرق (تحت الجلد)، داخل العضلة وعن طريق الفم.

نتج من اعطاء مادة PPD لكل من الكنسيات من فصيلة البنين (brown hisex) (PPD 100، 140 ملجرام/كيلوجرام) والفيروان من فصيلة البنين (PPD 70، 35 ملجرام/كيلوجرام) حدوث تغييرات في مكونات البلازما. كما ان هناك ارتفاع في تركيز البولينا وحمض البولوني. Aldolase، LDH، ALP، GPT، GOT ونشط الانزيمات والبوتاسيوم مصحوب بانخفاض في تركيز البروتين في البلازما. اضافة الى اضطرابات في نسب كل من الجلوكوز والدهون في البلازما.

عند دراسة الاثر الباثولوجي خلابة الكبد والكلئ عضلات القلب وعضلات الهيكل العظمي تبين حدوث تأضر في خلابة هذه الاتسحة مع وجود تغيير دهني إضافي إلى تزيف في بعض الأنسجة (العضلات) وانكماش في محفظة بومان في الكلئ. وذلك يشير الى اختلال وظائف كل من الكبد والكلئ وتأثر عضلات القلب. لوحظ ان هناك تغيير واضح في مكونات الدم تشير إلى حدوث فقر الدم نتيجة MCHC، MCV، MCH، TWBC Hb، PCV، RBC.

تعرض خلابة الدم لمادة الPPD ، تجدر الاشارة إلى حدوث الوفاة عند اعطاء جرعات كبيرة (PPD 100 - 140 ملجرام/كيلوجرام) للكلسيات و (PPD 70 - 150 ملجرام/كيلوجرام) للفيروان. وقد سبق ذلك ضعف شديد في عضلات الأطراف لما ادى إلى عدم القدرة على الهركة والوقوف.

اضافة الى تورم ظاهر في جبه العنق والراس مع حدوث تشنجات وضيق شديد في النفس.

عند اعطاء الكنسيات والفيروان جرعة واحدة من مادة الPPD لوحظ ان اقصى اثر سام يحدث للفيروان بعد مور 24 ساعة وفي الكنسيات بعد 72 ساعة. ثم متتابعة مدى قابلية اعطاء جسم الحيوان للشفاء من الاثر السام. وقد تبين ان الحيوانات تماثل للفيروان الا انه لم يحدث شفاء تام بعد مرور 120 ساعة.

عند اعطاء الحيوانات جرعة من مادة الPPD لمدة سته اسابيع بعدة جرعة واحدة في
الاسبوع (45 ملجم/كيلوجرام) للكثاكبيت (57.5 ملجم/كيلوجرام) للقبران. ظهرت التغييرات في البلازما والخلايا التي تشير إلى تأثر وظائف الكبد والكلى إضافة إلى فقر الدم. وذلك يؤكد أن التسمم يحدث نتيجة للتعرض المرمى للمادة.

عند تحري وجود مادة الصبغة بعد اعطاءها للحيوانات في كل من خلايا الكبد والكلى والدم.

تبين عدم وجود المادة مما يشير إلى حدوث تغيير كيميائي داخل خلايا جسم الحيوان.

اجريت دراسة لمعرفة الآثر الدوائي لمادة الPPD (صبغة الشعر) في عدة نسج موزعة من حيوانات التجربة. عند أضافة تركيز مخفض من محلول الصبغة لم يحدث آثار شاذ أو مضاد على الانسجة. كما لوحظ أن النسج تفقد حساسيتها تمامًا للاستيوي المحدودة للانقباض أو الانبساط بعد تعرضها لجرعات ذات تركيز عالي من مادة الصبغة. إلا أن أضافة محلول الصبغة لقلب الارنب الموزول قد صاحب ازدياد واضح في انقباض عضلات القلب. وقد لوحظ أن زيادة الانقباض يمكن إحصارها بإضافة محلول مضاد للحساسية قبل محلول الPPD مباشرة.

ما يشير الاعتقاد أن الآثر ناجح عن وجود مادة الهيستامين. عند حضانة مادة الصبغة في محلول يحتوي على أجزاء صغيرة من رئة المخنجر الغني فأن الملحق الناجح عن الحضانة يحدث انقباض واضح في عضلات اللثاتي الموزول من المخنجر الغني وقد ثم إحصار هذا الآثر بإضافة ماد مضاد للحساسية قبل إضافة الصبغة مباشرة. وعليه يمكن تفسير ذلك بأن مادة الصبغة تملك القدرة على تحرير مادة الهيستامين من الخلايا التي تحتوي على هذه المادة مثل خلايا القلب والرئة.