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Neuro and Nephro-Toxicity in Rats Topically Treated with Para-Phenylene Diamine

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Abstract: Para-phenylene diamine (PPD) systemic intoxication in Saudi Arabia has been increased over the last decade. The aim of the this study was to provide more insight into PPD intoxication with reviewing possible underlying mechanisms. The topical treatment with PPD (2mg/kg) for 5 weeks and its subsequent withdrawal caused decreased in monoamines (noerepinephrine, dopamine, serotonin and histamine) content in all brain region (cerebellum, brain stem, striatum, cerebral cortex, hypothalamus and hippocampus) at different time intervals. Moreover, the present results indicated that treatment with PPD for 5 weeks and its subsequent withdrawal caused increased in serum urea and serum creatinine levels. In conclusion. PPD causes serious multisystem toxicity and its selling to public should be officially restricted to reduce poisoning by this agent.

Key words: Paraphenylene diamine · Neurotransmitter · Histamine · Norepinephrine · Dopamine · Serotonin · Cereatinine · Urea · Rat

INTRODUCTION

Para-phenylene diamine (PPD) also called 1, 4 - diaminobenzene or 1,4 - phenylenediamine is an aromatic amine which has been used for dyeing furs photochemical measurements, accelerating vulcanization and azo-dye manufacturing, as well as for oxidizing hair dyes [1].

PPD is a derivative of paranitroanaline that is available in the form of white crystals when pure and rapidly turns to brown when exposed to air [2].

It is the main component of all black permanent hair dyes available on market, no matter the trade name. By default the darker the hair day is the higher concentration of PPD it has. Even the so called "natural" hair dyes contain PPD [3]. Several acute PPD poisoning cases had been reported, in particular, from Africa and Asia Where it is traditionally used mixed with Henna (leave of *Lawsonia alba*) tattoos which is traditionally applied to color the palms of hands and to dye the hairs. PPD added to henna past and after wards applied directly on to the skin. That way PPD assures the darkening of color (from orange to black) and prolonged lasting of the skin painting from 7 to 20 hours [4, 5]. First case of PPD poisoning was reported in a hairdresser in 1924 following exposure due to PPD dye handling [6].

The toxicity of PPD includes skin irritation, contact dermatitis, chemosis, lacrimation, exopthamlmose, or even permanent blindness, due to local contact. Ingestion of PPD produces two types of toxic effects. The first consists of rapid developmental of severe oedema of the face, neck, pharynx, tongue and larynx with respiratory distress, often requiring tracheostomy. In the later phase, rhabodomyolysis and acute tublar necrosis supervene [7-9]. Vomiting gastritis, hypertension, vertigo, tremors and convulsion have been reported [10, 11]. In addition to PPD dyeing properties, it was also used to kill wild animals when added to food [12]. Acute toxicity has been investigated following oral, subcutaneous, intraperitoneal and topical application in a variety of species. The LD_{50} following oral administration was 80-100 mg/kg in the rat, 290 mg/kg in mice, 250 mg/kg in rabbit and 100 mg/kg in cats [13]. The lethal dose for human was estimated to be 10 gram of pure PPD [14].

Since the tattoo industry is not regulated, people are still getting black henna tattoos and exposing themselves to serious toxicity problem. In addition tattoos are very popular in Arabian Golf, especially in Saudi Arabia and there is no report has been available regarding chronic dermal exposure and subsequent neurotoxicity by PPD. The present investigation was therefore aim at establishing the role of PPD in hair dye mediated neurotoxicity and toxicological effect on kidney impairment function with reviewing the possible underlying mechanisms.

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MATERIALS AND METHODS

Chemicals: Para-phenylene diamine (PPD) was bought from the open market in Saudi Arabia (Jeddah).

Toxicological Study: The study was conducted during July -October of 2010 in the Department of Zoology, King Abdul Aziz University in Saudi Arabia (Jeddah). Seventy two of male rats (*Rattus norvegicus*) weighing (140+10 g) were during the present study. The animals were housed in cages under ambient temperature of 21+ 3°C and 12:12 h of L:D cycle. The animals were acclimatized to the laboratory condition for one week at the commencement of the treatment protocol.

The rats were randomly divided into two groups. The first group (n=36) was divided into six subgroups each of 6 rats, each animals were painted on their dorsal side clipped free of fur with 2mg/kg b.w. of PPD dissolved in double distilled water for 10 minutes. The second group (n=36) was divided as the first group receive distilled water painted on their dorsal said as in other PPD treated animals and served as control. The animals were painted for five continuous weeks with PPD solution or vehicle alone according to Bharali and Dutta [15].

One subgroup (6 animals) was decapitated at the end of each week up to 5 weeks. To examine the withdrawal effect, the remaining subgroup was decapitated after one week from the withdrawal of PPD. At the end of treatment, the rats of both control and experimental groups were sacrificed and blood collected for kidney biochemical function were determined including urea [16] and creatinine [17]. The brain was rapidly dissected and separated into two equal halves. Each half was then separated into the following regions according to the Glowinski and Iversen [18] method: cerebellum, brain stem, striatum, cerebral cortex, hypothalamus and hippocampus. The brain tissues were wiped dry, weighed and wrapped into quickly plastic frozen in dry ice pending analysis. noerepinephrine (NE), dopamine (DA) and serotonin (5-HT) were extracted and estimated according to the Chang [19] method and modified by Ciarlone [20] (1978). Histamine (HA) was estimated according to the method described by Shore *et al*. [21]. The fluorescence was measured in Jenway 6200 fluorometer.

Statistical Analysis: Values reported are means ± SEM. Differences between means were estimated by the Student'*t* test using SPSS program V.14. Percentage difference is representing the percent of variation with respect to the control. Probability (P) given. P less than 0.05 was considered significant.

RESULTS

Figures (1-5) and Table 1 illustrate the impact changes of topically treatment of PPD induced on brain regions and kidney function.

As shown in Fig. 1 topical /kg b.w for 5 weeks caused changes of NE content in different brain regions of male albino rats. After one week, PPD caused a significant decrease of NE content in brain stem and hypothalamus.

Table 1: Frequency of symptoms observed in male albino rats treated locally with para-phenylene diamine (2 mg/kg)

Clinical symptoms	Percentage
Swelling face and neck	98%
Dark discoloration of urine	100%
Ataxia	65%
Vomiting	10%
Seizure	15%
Mortality	5%

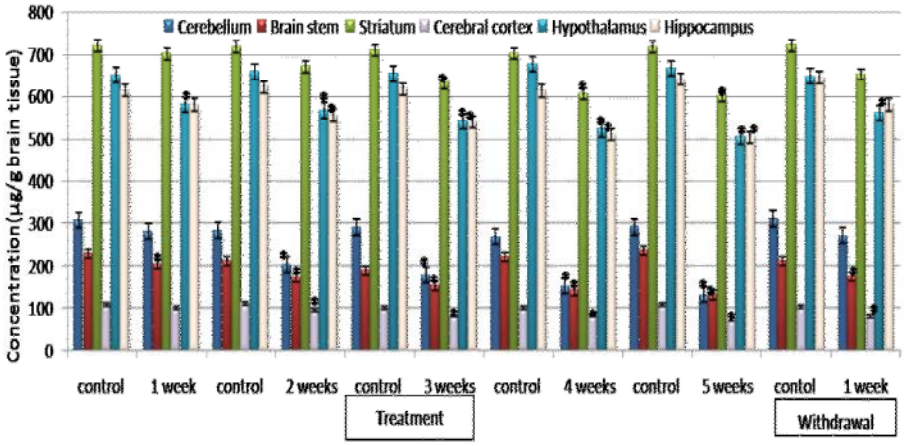

Fig. 1: Effect of topically treatment of para-phenylene diamine (2 mg/kg) and its subsequent withdrawal on dopamine (NE) content in different brain regions of male albino rats. * P < 0.05

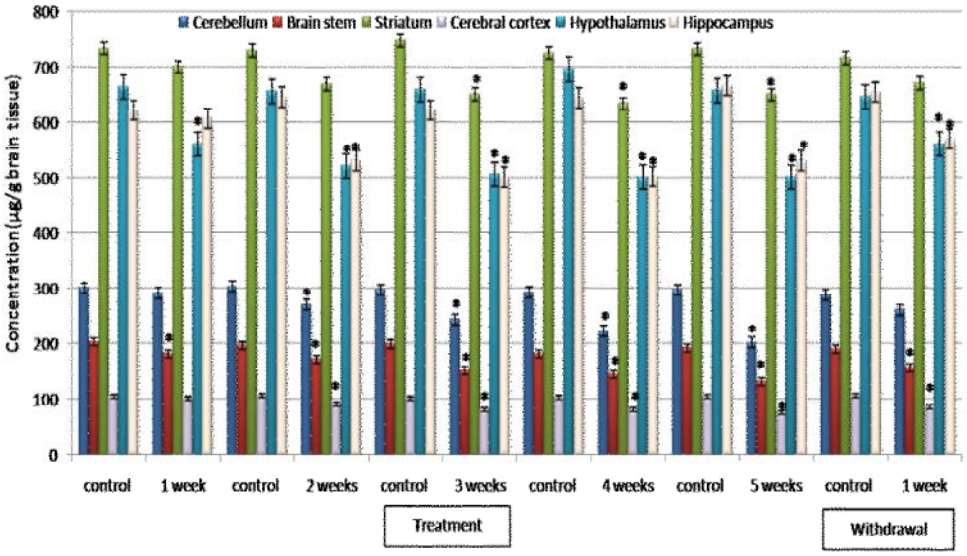

Fig. 2: Effect of topically treatment of para-phenylene diamine (2 mg/kg) and its subsequent withdrawal on dopamine (DA) content in different brain regions of male albino rats. * $P < 0.05$

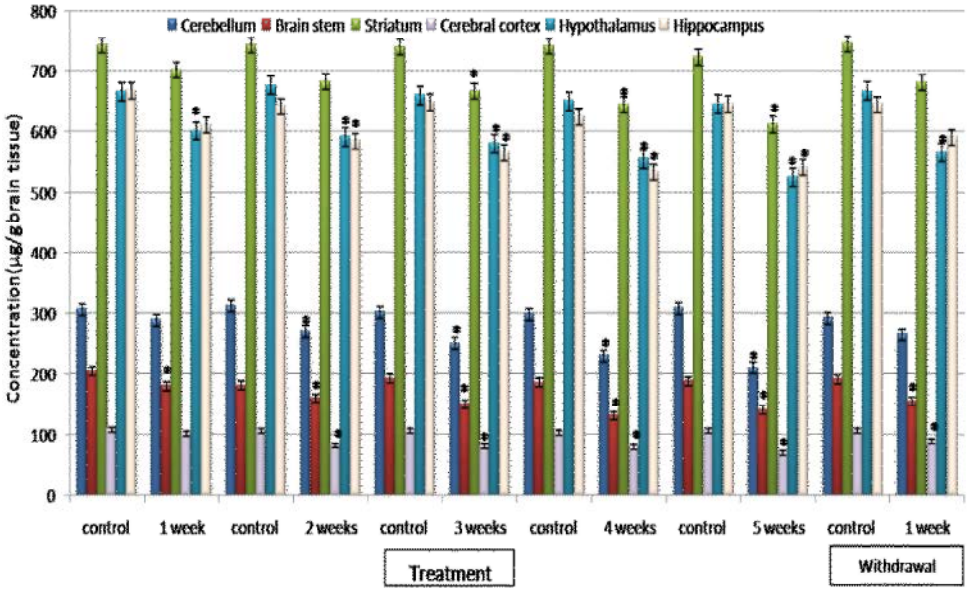

Fig. 3: Effect of topically treatment of para-phenylene diamine (2 mg/kg) and its subsequent withdrawal on Serotonin (5-HT) content in different brain regions of male albino rats. * $P < 0.05$

After two weeks, PPD caused a significant decrease in all tested brain regions except in the striatum, it caused an insignificant change. Treatment of PPD caused a significant decreased of NE content in all tested brain regions after 3, 4 and 5 weeks. On other hand, after one week of the withdrawal of PPD a significant decreased of DA content in brain stem, cerebral cortex and hypothalamus. while there was an insignificant changes in the other tested brain regions were record.

Data in Fig. 2 shows that treatment with PPD produced changes of DA content in different brain regions of albino rat after one week. PPD caused a significant decrease of DA content in brain stem and hypothalamus. After two weeks PPD caused a significant decreased of DA content in all tested brain regions except in striatum, it caused in significant changes. There was a significant decrease in DA content in all tested brain regions after 3, 4 and 5 weeks. The significant decrease in DA content persisted one week of the withdrawal in brain stem, cerebral cortex, hypothalamus and hippocampus.

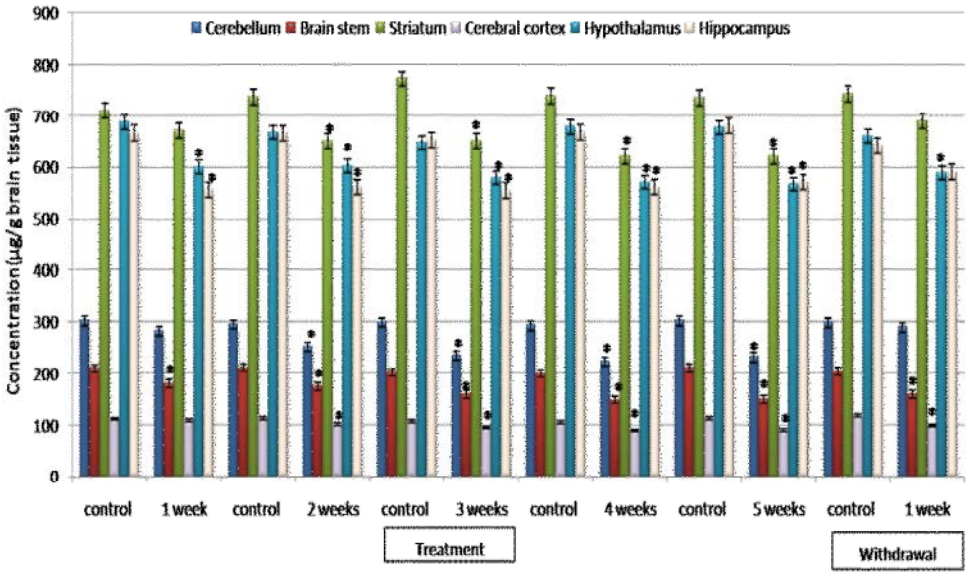

Fig. 4: Effect of topically treatment of para-phenylene diamine (2 mg/kg) and its subsequent withdrawal on histamine (HA) content in different brain regions of male albino rats. * $P < 0.05$

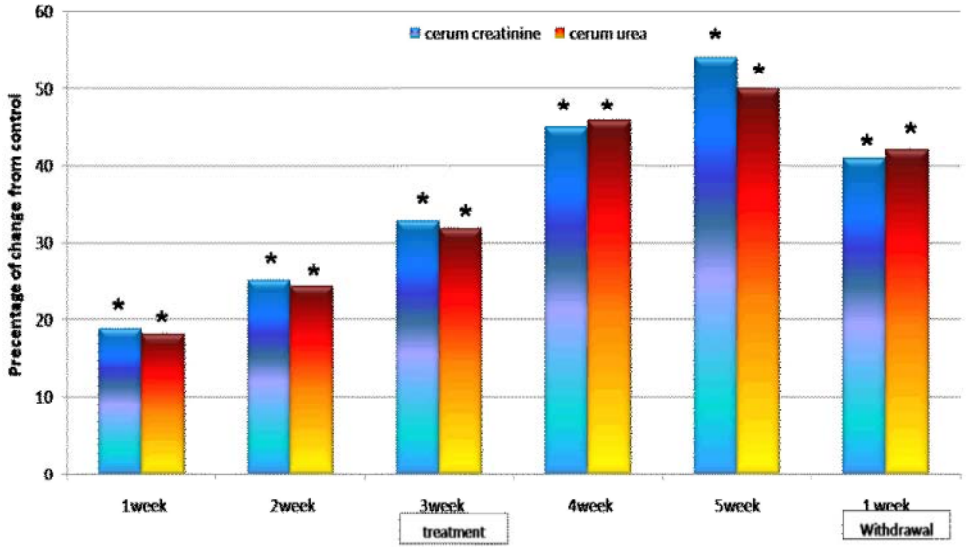

Fig. 5: Effect of topically treatment of para-phenylene diamine (2 mg/kg) and its subsequent withdrawal on serum creatinine and serum urea of male albino rat. *P<0.05

Fig. (3) shows that topical treatment with PPD (2 mg/kg) induced a significant decrease in 5-HT content in brain stem and hypothalamus after one week and a significant decrease in 5-HT content after two weeks in all tested brain regions except in striatum. The 5-HT content still significantly decrease after 3, 4 and 5 weeks in all tested brain regions. The withdrawal of PPD caused a significant decrease of 5-HT content in brain stem, cerebral cortex and hypothalamus.

Moreover, treatment of PPD significantly decreased the HA content in brain stem, hypothalamus and hippocampus after one week. The HA content still significantly decrease after 2, 3, 4 and 5 weeks in all tested brain regions. The HA content remained significantly decreased after one week of the withdrawal in brain stem, cerebral cortex and hypothalamus.

In serum, a significant increase was observed at 1, 2, 3, 4 and 5 weeks in urea and creatinine levels (mg/dl) after treatment with PPD (2 mg/kg). Moreover highly significant increase in week 5 when compared with control group. Urea and creatinine levels remained significantly increased after one week of the withdrawal.

Table 1 Shows that topical treatment with para-phenylene diamine (2mg/kg) caused: swelling face and neck (98%), dark discoloration of urine (100%), ataxia (70%), seizure (15%) and mortality (5%) of total rats treatment.

DISCUSSION

The toxicity of PPD is a multisystem involvement [9, 22]. The toxicity of PPD occurs through skin absorption. A number of earlier report suggested that a fraction of topically applied PPD alone or in combination with an oxidizing agent reach systemic circulation after percutaneous absorption [23]. PPD thought to be oxidized *in vivo* to quinine diamine and then acetylated to form a diacetyl derivative. The quinine diamine of PPD is the toxic derivative [24 -26].

Experimental studies in guinea pigs when dermally exposed to PPD revealed that, PPD is absorbed through the skin into serum and excreted in urine. There was an increase in malon dialdehyde (MDA), which indicates lipid peroxidation, suggesting that increased free radical formation is responsible for histopathologically tissue damage in many organs [27]. Also it was believed that the PPD toxicity is due to altered vascular permeability and involvement of the nervous system [28].

From the present result it was clear that the treatment of PPD caused a significant decreased in monoamines (NE, DA, 5-HT and HA) content most of the tested brain regions at the different time intervals. After the withdrawal of PPD, there are regional difference in the effect. On other hand, treatment with PPD caused a significant increased in Urea and creatinine levels. These changes associated with swelling face and neck, Dark discoloration of urine,ataxia, Seizure and mortality.

These findings were matched with Mathur *et al*. [29] who demonstrated that, at high concentration and long PPD exposure period cell death produced. This effect together with lipid peroxidation can be the cause of the production of superoxide and hydrogen peroxide by the PPD auto-oxidation. Several lines of evidence indicated that increased free in PPD poisoning radical formation may be responsible for the deleterious tissue damage observed in animal studies [30-32].

Free radicals play an important role in several pathological conditions of the central nervous system (CNS) where they directly injure tissue. Free radicals produce tissue damage through multiple mechanisms, including excito-toxicity, metabolic dysfunction, and disturbance of intracellular homeostasis of calcium. 3. Oxidative stress can significantly worsen [34-36]. Moreover, free radicals caused neural sensitization damage lipid membranes, with disproportionate damage and loss of omega 3 essential membrane lipids. This lipid damage impairs the brain and nerve cell coating (myelin).

It also damages function of cell membranes and membranes of mitochondria (energy production), ribosomes (which make proteins, enzymes) DNA (genetic material), membrane receptor sites (hormones, etc.) cell messenger sites to communicate with other cells and body organs and cell death induced by DNA fragmentation and lipid peroxidation [14, 35, 37]. This oxidative damage/stress associated with reactive oxygen species (ROS) is believed to be involved in the pathophysiological role in aging of skin and several diseases like heart disease, shock, chronic inflammatory diseases, neurodegenerative disorders including mental alteration, production of nitric oxide and vascular damage [36, 38, 39, 40].

Moreover free radical cause hypoxic or ischemic state deals with excitatory amino acid (EAA) aspartate (ASP) and glutamate (GLU), synaptically released transmitter which are endowed with neurotoxic effects [41-43]. Derangements in glutamate neurotransmission have been implicated in several neurodegenerative disorders. Activation of the N-methyl-D-aspartate (NMDA) receptor subtype of glutamate receptors results in the influx of calcium which binds calmodulin and activates neuronal nitric oxide synthase (nNOS), to convent L-arginine to citrulline and nitric oxide (NO). NO has many roles in the central nervous system as a messenger molecule, however, when generated in excess NO can be neurotoxic. Excess NO is in part responsible for glutamate neurotoxicity in primary neuronal cell culture and in animal models of stroke. Increasing evidence indicates that many neurologic disorders may have components of free radical and oxidative stress induced injury [44]. Hayashi *et al*. [45] and Gilman *et al*. [46] reported that free radicals induce sodium-calcium exchange, which increase intracellular calcium concentration and consequently increase neurotransmitter release.

From the previous studies and present results, it could be concluded that the chronic treatment of Pa-raphenylene diamine caused decrease in monoamines content in different brain regions which may be due to increased free radical formation in PPD poisoning which may be responsible for neurodegenerative disorders, vascular damage, excitatory amino acid released and increase intracellular calcium concentration as a result the content of monoamines are decreased.

From the curent results, it is also clear that The most affected regions after the withdrawal of PPD are: brain stem, cerebral cortex and hypothalamus.

Brain stem which is the brain region responsible for basic vital life functions such as breathing, heartbeat, blood pressure essential and eye movement. These result coincide with those in animal study, It was reported that 89% of the mice fed PPD developed lenticular changes indicating that PPD has cataractogenous effects, which are related to the duration, amount and individual sensitivity. Exophthalmia and permanent blindness due to optic nerve atrophy following PPD poisoning were reported [14, 47, 48]. In addition Dyspnoea, tachypnoea and asphyxia with chest pain following acute PPD poisoning have been reported in a number of studies [48, 49]. PPD was proved to be the cause of asthmatic attacks in the sensitive individuals. Difficult in breath to also has been reported [14, 49]. Moreover, In many reports of PPD toxicity cardiac arrest was the main cause of death. In these cases cardiac arrest is attributed to arrhythmia. Cases of myocardial infarction associated with cardiac rhabdomyolysis have been reported [50].

Cerebral cortex which is the brain regions responsible for motor. Pervious study demonstrated that PPD induce skeletal muscle toxicity. Saito *et al*. [51] reported that Skeletal muscle biopsy of patients showed scattered coagulation necrosis and inflammatory cellular infiltration. Foot drop palatopharyngeal and laryngeal paralysis were also reported [52].

Hypothalamus which is the brain regions responsible for food and water intake regulation, body temperature and water balance. Kallel *et al*. [12] proposed that PPD-intoxication induce muscle edema causing pressure on blood vessels and decrease blood flow, resulting in damage to the muscle. Various investigators have been previously demonstrated that the kidney are particularly vulnerable to effects of noxious agents because of their high perfusion rate [33, 48]. Renal lesions associated with PPD intoxication received much attention because most of the clinical investigators reported renal failure. However, evidence of sever nephrotoxicity has been reported in humans. Laboratory investigations reveled elevated serum creatinie and Urea as a result of kidney injury [11]. From the present results it is also clear that the treatment of PPD caused a significant decreased in Urea and creatinine levels in serum. These results are in agreement with previous study. Laboratory investigations reveled elevated serum creatinine and Urea which may be due to kidney injury. The metabolic products of PPD have a high urinary excretion rate and their oxidation produces quinine-diamine, which is a potentially nephrotoxic substance. Autopsy of patients revealed renal tubular occlusion due to myoglobin casts with histological evidence of acute tubular necrosis [53, 54].

From the previous studies and the present results, it could be concluded that the treatment of PPD caused change in urea and creatinine levels which may be in part, due to the presence of quinine-diamine which caused kidney injury, at the same time the presence of free radical which caused neurotoxicity in the hypothalamus; as a result the level of urea and creatinine in serum is increased.

Pervious changes in monoamines content, urea levels and creatinine levels in present study associated with swelling face and neck, Dark discoloration of urine, ataxia, vomiting, Seizure and mortality. These results are in agreement with the previous study which indicate that clinical manifestation of systemic PPD intoxication were associated with oedema of the face, neck, pharynx, tongue, chocolate brown colour of the urine, muscle necrosis, vomiting, convulsions, coma and sudden cardiac death [12, 29, 33]. The symptoms are considered to be dose related and patients with ingestion of large amounts of PPD have higher morbidity and mortality [9, 55].

CONCLUSION

Considering the result observed in the present investigation, further experimental information it is clear that treatment of PPD caused neurotoxicity this is may be due to increased free radical formation in PPD poisoning which may be responsible for neurodegenerative disorders, vascular damage, excitatory amino acid released and increase intracellular calcium concentration as a result the content of monoamines is decreased, at the same time PPD caused nephrotoxicity which may be in part, due to the presence of quinine-diamine which caused kidney injury, at the same time the presence of free radical which caused neurotoxicity in the hypothalamus; as a result the level of urea and creatinine in serum is increased. PPD causes serious multisystem toxicity. So, a program of public education with official restriction of PPD selling to the public should be considered. All cosmetics,including hair dye products that are sold in the retail market must have their ingredient listed on the label to reduce poisoning by this agent.

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