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RESEARCH ARTICLE

Prophylactic Effects of Methylene Blue, Coconut and Olive Oils Supplements on Hemato-Biochemical and Histo-pathological Parameters against *p*-Phenylenediamine Toxicity in Male Albino Rats

Majeeda Rasheed[1$], Muhammad Arfan Zaman[2$], Aqdas Zafar[3#], Muzaffar Ali Khan[4#], Shazia Anjum[3,5]*, Hafiz Muhammad Ali[6]**, Shafqat Hussain[3], Moazzama Zafar[3], Jamila Yasin[3], Riaz Hussain[6] and Atiq Ali[6]

[1]Department of Life Sciences, Khawaja Fareed University of Engineering and Information Technology (KFUEIT), Rahim Yar Khan, Pakistan
[2]Department of Pathobiology, College of Veterinary and Veterinary Sciences, Jhang, Pakistan
[3]Institute of Chemistry, Faculty of Chemical and Biological Sciences, the Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan
[4]Department of Microbiology and Molecular Genetics, Bahauddin Zakariya University Multan-60800, Pakistan
[5]H.E.J. Research Institute of Chemistry, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan
[6]Faculty of Veterinary and Animal Sciences, the Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan
[$]Majeeda Rasheed and Muhammad Arfan Zaman contributed equally to this work
[#]Aqdas Zafar and Muzaffar Ali Khan contributed equally to this work
*Corresponding authors: shazia.anjum@iub.edu.pk; hmali_uaf@iub.edu.pk

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A B S T R A C T

Self-poisoning with *p*-phenylenediamine (PPD) is a common method in individuals experiencing social or economic stress. The current study was planned to determine various physiological effects of the poison and to identify an effective antidote against PPD toxicity. The hemato-biochemical profile along with histo-pathological examination of kidneys, brain, heart and lungs were conducted in the adult male albino rats (n=25) supplemented with methylene blue, coconut and olive oils post-PPD toxicity. A significant ($p<0.05$) decrease in the hematological parameters except a significant ($p<0.05$) increase in the leukocyte count was observed in the animals injected only with PPD (30mg/kg BW) compared to the control negative group. The animals supplemented with methylene blue, olive, or coconut oils post-PPD injection showed a significant ($p<0.05$) amelioration in the hematological parameters compared to the PPD-only control group. PPD toxicity significantly ($p<0.01$) increased the hepatic (ALT, AST, bilirubin) and renal (urea, creatinine) markers and level of CK-MB compared to the control negative group. The supplementation of animals with methylene blue, coconut and olive oils showed protective effects by significantly ($p<0.05$) decreasing the levels of hepatic and renal markers and CK-MB along with improvement in the histo-architecture of the heart, kidneys, lungs and brain. The animals supplemented with olive oil showed better results, since it has been found to partially reverse the effects of PPD toxicity compared to methylene blue and coconut oil and thus, could be used as a possible anti-dote against PPD toxicity.

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INTRODUCTION

A poison is a natural or synthetic compound that adversely affects the physiological functions of the body through chemical reactions. It causes damage or dysfunction to the living tissues and produces fatal outcomes in the body, whether it is ingested, inhaled, absorbed through the skin, or injected parenterally (Karki and Risal, 2012). Commonly used toxins, if exposed accidentally to marine or terrestrial organisms, not only cause adverse fatal outcomes but also reduce the life span of the affected species (Adoho *et al*., 2022; Akram *et al*.,

2022; Malik *et al*., 2022). Self-poisoning is one of the significant causes of death worldwide (Staniszewska *et al*., 2022) and different types of chemicals are used for this purpose like wheat pills (Zn or aluminum phosphide), pesticides, analgesics and anti-depressants (Safdar *et al*., 2021). Once entered the body, the chemical leads to local or general injurious medical effects that lead to physiological disability or ultimately death of the individual. The agent used to decrease or cope with the effects of a poison is an antidote and the lack of an appropriate and timely therapy by an antidote makes it a lethal suicide attempt. According to the report of the World Health Organization (WHO), more than 0.7 million people die each year by suicide and the rate is constantly increasing each year (WHO, 2021).

p-phenylenediamine (PPD); commonly known as Kala Pathar (black stone) is one of the commonly used chemicals in ink and rubber industries, is easily available for attempting suicide and has a high mortality rate of up to 80% (Sayed *et al*., 2024). The cases of PPD toxicity are reported globally but the ratio of poisoning is higher in developing countries like India, Sudan, Tunisia, Morocco, Egypt and Pakistan (Ababou *et al*., 2000; Abdelraheem *et al*., 2009; Mahsud 2015; Mustafa, 2001). In the early 90s, PPD poisoning was the leading source of self-harm in Morocco and 374 cases of PPD poisoning were reported at the National Poison Control Center of Morocco during 1992-2002 (Filali *et al*., 2006). Similarly, several cases of PPD poisoning have been reported in India (Jain *et al*., 2011; Prabhakaran, 2012), Morocco (Filali *et al*., 2006), Egypt (Sayed *et al*., 2024), Sudan (Abdelraheem *et al*., 2009; 2010), UAE (Bhagavathula *et al*., 2019) and Pakistan (Khan *et al*., 2018; Asghar *et al*., 2022). In Pakistan, 11 studies have reported the use of PPD for self-poisoning in different regions of the country (Sultan *et al*., 2020; Safdar *et al*., 2021).

When skin is exposed to PPD through tattooing or dying the hair, it could cause severe skin allergy. Intended or accidental oral intake of PPD causes nausea, rhabdomyolysis, cervico-facial edema and acute renal failure (Elgassim *et al*., 2022). Due to swelling of the neck due to cervical edema, the victim feels uncomfortable breathing. At the oral intake of PPD, it is rapidly absorbed by the mucus membrane of the gastrointestinal tract and is metabolized into benzoquinone-diamine which is a cytotoxin (Senthilkumaran and Jena, 2019). For detoxification, it is acetylated in two major metabolites; N-acetyl-PPD and N, N-diacetyl-PPD and is excreted in the urine (Senthilkumaran and Jena, 2019). Since no specific antidote for PPD is available to date, the treatment of the affected person is usually done by supportive therapy including gastric lavage and correction of electrolyte imbalance and acidosis along with the use of methylene blue for the treatment of met-hemoglobinemia (Kamaloddini *et al*., 2021). Previously, gastric lavage with sodium bicarbonate and coconut oil has been done in patients with zinc phosphide poisoning (Altintop and Tatli, 2017). Oleuropein, a component of olive oil was found to be an effective antioxidant (Cicerale *et al*., 2012) and olive oil was used to ameliorate arsenic-induced hepatotoxic effects (Cheema and Ali, 2018). To reduce the toxic effects of PPD, treatments like emergency oxygen inhalation, tracheostomy and hemodialysis are given to the victim of PPD toxicity. Hence, the objectives of the study are to evaluate the hematological, biochemical and cellular damages after PPD toxicity and sort out a possible antidote against PPD poisoning.

MATERIALS AND METHODS

Animal management and experimentation: Adult male BALB/c albino rats (n=25) were kept at 25ºC for 3 weeks before the start of the experimental trial for acclimatization. The animals had free access to standard food (purified rat diet; crude protein=18-22%, crude fiber=3-5% and 2750 Kcal/Kg of metabolizable energy) and clean filtered drinking water was available ad libitum. The rats were divided into five groups (5 rats/group) wherein, group A was treated as control negative i.e. neither PPD nor any treatment was given. The toxicity was induced in the rest of the groups (B to E) through intra-peritoneal injection (30mg/kg body weight; BW) of PPD. Group B was given PPD only, to check its toxic effects and hence, was regarded as a PPD-only control group. Groups C, D and E were given either methylene blue (2mg/kg BW), coconut oil (5ml/kg BW) and olive oil (2ml/kg BW) respectively, after 10 min of PPD injection. The behavioral alterations were continuously noted in the treated rats during the whole experimental period before and after the treatments. At 72 hrs, the rats of all the groups were sacrificed by CO_2 inhalation and all efforts were made to minimize the animal suffering (Ali *et al*., 2014; Urbinati *et al*., 2016). The blood samples were collected for hematological and serum biochemical analysis and the tissue samples of different organs were collected and preserved in 10% formalin solution until further histo-pathological studies. The animal ethical committee of The Islamia University of Bahawalpur granted consent for all the animal experimentations for the study (IUB/AEC-2022-749, Dated: 02-12-2022).

Determination of blood and serum biochemical profiles: The blood was either collected in EDTA tubes for hematological studies or in EDTA-free tubes for serum biochemical analysis. The serum was separated from the collected blood and preserved at -20ºC until further analysis (Qureshi and Ali, 2016; Ayub *et al*., 2018; Malik *et al*., 2018). For hematology, total erythrocyte count (RBCs), leukocyte count (WBCs), hemoglobin (HB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and neutrophils (NEUT) count were determined by using Sysmex. The serum was analyzed to determine creatinine kinase-MB (CK-MB) as the cardiac marker; alanine transaminase (ALT), aspartate transaminase (AST) and total bilirubin as the hepatic marker and urea and creatinine as renal markers by using commercially available standard kits (Al-Farraj *et al*., 2024).

Histo-pathological examination of tissue samples: For histo-pathological examinations,0.5–1.0cm thick pieces of kidneys, brain, heart and lungs were collected and immediately fixed in freshly prepared 4% v/v formaldehyde solution for 48h at 4°C under agitation (Luqman *et al*., 2020; Ali *et al*., 2024). These tissues were processed by paraffin sectioning technique and 4–5µm

thick histological sections were stained by Hematoxylin and Eosin staining techniques, as previously described (Ali *et al*., 2017; Sikandar *et al*., 2020; Al-Farraj *et al*., 2024).

Statistical analysis: The collected data were presented as mean±SE. One-way analysis of variance (ANOVA) was used for the statistical analysis by using IBM SPSS statistics (version 20). Post-hoc Tukey's test was conducted to compare the various parameters between the control and the treated animals. $p<0.05$ was considered a statistically significant level.

RESULTS

Analysis of PPD through FTIR spectroscopy: Fourier-transformed infrared (FTIR) spectroscopy characters were used to analyze the presence of functional groups in PPD. The standard FTIR spectrum of PPD (Fig. 1) was compared with that of our experimental compound (PPD) (Fig. 2) and the values of IR peaks of PPD used in the experiment and standard PPD are given (Table 1).

Effects of treatments on hematological parameters against PPD toxicity: There observed a significant decrease in RBC count ($p<0.05$), HB concentration ($p<0.05$), HCT ($p<0.01$), MCV ($p<0.05$) and MCH ($p<0.05$) and a significant ($p<0.01$) increase in the leukocyte count in the animals (group B) injected with PPD (30mg/ Kg) compared to rats of control negative group A. The animals supplemented with methylene blue post-PPD injection showed a significant ($p<0.05$) increase in HB, HCT and MCV and a significant ($p<0.05$) decrease in WBCs compared to the animals of PPD-only control group B. Similarly, the coconut and olive oils also had significant ($p<0.05$) prophylactic effects on the hematological parameters of the animals of groups D and E (Table 2).

Effects of treatments on hepatic enzymes against PPD toxicity: PPD toxicity significantly ($p<0.01$) increased the hepatic markers (ALT, AST, Bilirubin) compared to the control negative group A. Olive oil has great protective effects against PPD toxicity since it significantly ($p<0.01$) decreased the level of hepatic markers compared to PPD-only control group B (Table 3). While both coconut oil and methylene blue also significantly ($p<0.05$) decreased the level of these enzymes compared to the PPD-only control group (Table 3).

Table 1: Relative wave number of standard PPD and experimental PPD sample.

Sr. No.	Functional group	Wave number (cm^{-1})	
		Standard PPD	Sample PPD
1	Disubstituted ring	831	822
2	C-N-C bending	1263	1257
3	c-c stretching	1516	1506
4	C-H stretching	3009	3196
5	N-H stretching	3375	3371

Effects of treatments on renal and cardiac markers against PPD toxicity: The level of urea and creatinine significantly ($p<0.01$) increased in the serum of rats of group B which were injected only with PPD (30mg/kg) toxicity (PPD-only control group) compared to control negative group A. The rats supplemented with different treatments significantly decreased the levels of creatinine ($p<0.01$) and

Table 2: Effects of supplementation of methylene blue, coconut oil and olive oil on the hematological profile of albino rats exposed to PPD

Parameters	Groups				
	A	B	C	D	E
WBC (10^3µl)	12.10±0.18	18.10±0.13*	16.03±1.15*	16.10±1.11*	14.19±1.14*
RBC (10^6µl)	10.20±0.01	6.10±0.01*	7.26±2.16	8.25±0.67*	8.87±0.14*
HB (g/dl)	14.90±0.20	8.70±0.12*	10.20±1.63*	11.40±0.93*	11.63±1.03*
HCT (%)	52.50±1.25	38.50±1.17**	41.05±1.22*	42.90±1.85*	45.05±1.10*
Neutrophil (%)	38.40±0.25	95.40±0.18**	89.70±1.09*	91.80±2.29	73.80±3.38**
MCV (fl)	51.50±0.22	35.50±1.17*	39.10±0.89*	37.60±1.12*	36.20±2.48
MCH (pg)	14.70±0.12	9.70±0.178*	10.16±0.42	10.28±0.36	10.90±1.80*

PPD injection significantly ($p<0.05$) decreased the number of RBCs and HB concentration while significantly ($p<0.05$) increased the number of leukocytes compared to control negative group A. Different treatments post-PPD injection significantly ($p<0.05$) increased the number of RBCs and level of HB, HCT, MCV and MCH and significantly ($p<0.05$) decreased the number of leukocytes and neutrophils compared to PPD-only control group B. *=$p<0.05$, **=$p<0.01$. Group A: control negative, Group B: PPD-only control, Group C: PPD injection and supplemented with methylene blue, Group D: PPD injection and supplemented with coconut oil, Group E: PPD injection and supplemented with olive oil.

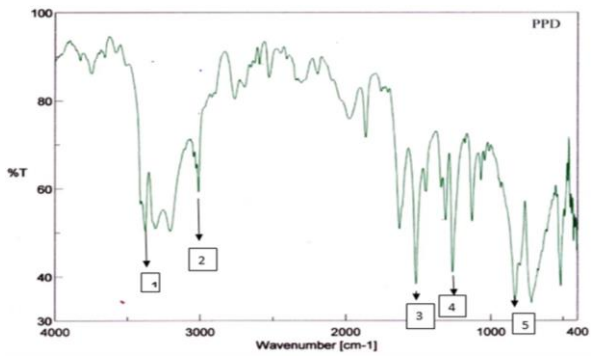

Fig. 1: Standard FTIR spectrum of standard PPD. Peak-1 at 3375/cm shows N-H stretching of the amine group, peak-2 at 3009/cm shows C-H stretching, peak-3 at 1516/cm shows C-C stretching, peak-4 at 1263/cm shows C-N-C bending and peak-5 at 831/cm is the peak of the disubstituted ring.

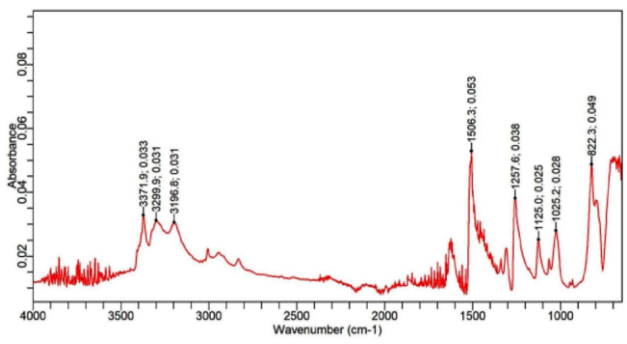

Fig. 2: FTIR spectrum of our experimental PPD. Peak-1 at 3371.9/cm shows N-H stretching of the amine group, peak-2 at 3196.8/cm shows C-H stretching, peak-3 at 1506.3/cm shows C-C stretching, peak-4 at 1257.6/cm showed C-N-C bending and peak-5 at 822.3/cm is the peak of the disubstituted ring.

Table 3: Effects of supplementation of methylene blue, coconut oil and olive oil on serum biochemical profile of albino rats exposed to PPD

Parameters	Groups				
	A	B	C	D	E
AST (U/L)	12.05±0.18	28.01±1.13**	17.03±1.55*	16.05±1.81*	14.04±0.64**
ALT (U/L)	15.07±1.01	26.04±1.21**	19.01±1.14*	18.06±0.67*	16.07±1.16**
Bilirubin (mg/dl)	0.98±0.20	2.10±0.12**	1.29±0.63*	1.54±0.93*	1.21±0.73*
Urea (mg/dl)	17.01±1.25	31.01±2.17**	29.01±1.22*	27.3±1.85*	23.02±1.10**
Creatinine (mg/dl)	0.35±0.05	1.80±0.18*	0.67±0.05*	0.59±0.09*	0.39±0.08**
CK-MB (U/L)	29.23±1.40	50.11±1.22**	44.03±1.16	41.01±2.01*	33.09±2.53**

PPD injection significantly increased the level of hepatic ($p<0.01$) and renal ($p<0.01$) enzymes and CK-MB ($p<0.01$) compared to control negative group A. Different treatments post-PPD injection significantly decreased the level of hepatic ($p<0.05$) and renal ($p<0.01$) enzymes and CK-MB ($p<0.05$) compared to PPD-only control group B. *=$p<0.05$, **=$p<0.01$. Group A: control negative, Group B: PPD-only control, Group C: PPD injection and supplemented with methylene blue, Group D: PPD injection and supplemented with coconut oil, Group E: PPD injection and supplemented with olive oil.

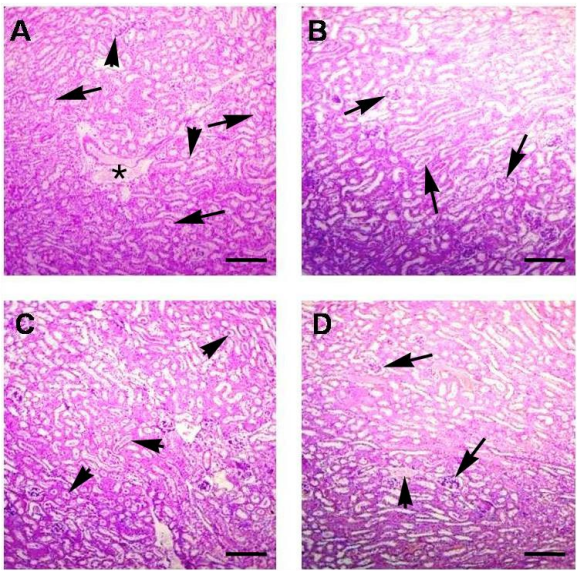

Fig. 3: Photomicrograph of the effects of supplementation of methylene blue, coconut oil and olive oil on histo-pathological lesions in the kidneys of albino rats exposed to PPD. A) necrosis of tubular cells (arrow heads), degeneration of renal tubules (arrows) and edema (*). B) increased urinary space (arrows)was increased in the kidneys of animals of group B which was relatively decreased with the addition of different supplements, especially in the group supplemented with olive oil. C) Necrosis of tubular cells, degeneration of renal tubules (arrowheads), deterioration of glomeruli and congestion also decreased with different treatments with more pronounced results observed in group D. D) edema (arrow head) and deterioration of renal tubules (arrows). A: PPD-only control, B: PPD injection and treated with methylene blue, C: PPD injection and treated with coconut oil, D: PPD injection and treated with olive oil. Scale bar = 50µm, 400X, Stain: Hematoxylin and Eosin

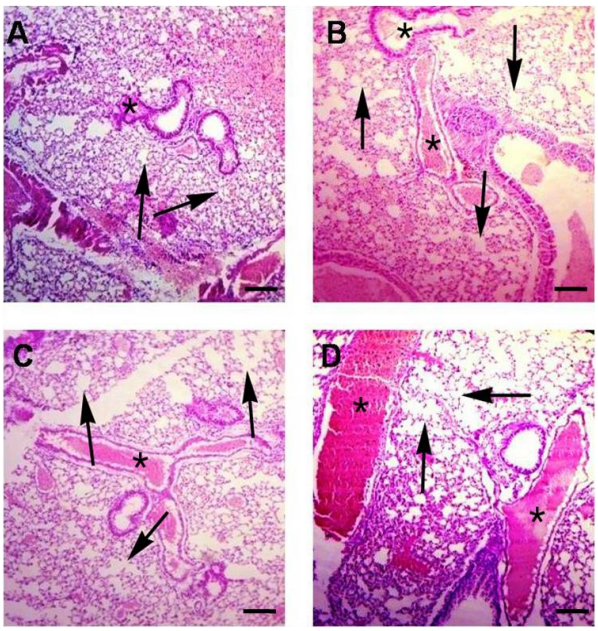

Fig. 4: Photomicrograph of the effects of supplementation of methylene blue, coconut oil and olive oil on histo-pathological lesions in the lungs of albino rats exposed to PPD. Inflammatory materials, hemorrhages (*) and interstitial pneumonia (arrows) were found to be relatively decreased in the groups treated with coconut and olive oil compared to the PPD-only control group. Atelectasis (collapse of pulmonary alveoli, hemorrhages (*) and interstitial pneumonia (arrows); C) hemorrhages (*) and interstitial pneumonia (arrows) and D) hemorrhages (*) and interstitial pneumonia (arrows) was also found to be significantly decreased in different supplementary treatments compared to the control group. A: PPD-only control, B: PPD injection and treated with methylene blue, C: PPD injection and treated with coconut oil, D: PPD injection and treated with olive oil. Scale bar = 50µm, 400X, Stain: Hematoxylin and Eosin

urea ($p<0.01$) compared to those in the animals of group B (Table 3). The effects of olive oil on urea ($p<0.01$) and creatinine ($p<0.01$) were found to be significant compared to the other two treatments (methylene blue and coconut oil) to counter the effects of PPD toxicity. The level of CK-MB increased significantly ($p<0.01$) in the rats of the PPD-only control group compared to the control negative group A. While, the rats treated with methylene blue ($p<0.05$), olive ($p<0.01$) and coconut ($p<0.05$) oils showed a significant decrease in CK-MB level post-PPD injection (Table 3) compared to PPD-only control group B.

Histo-pathological effects of treatments against PPD toxicity: The histo-pathological tissues of kidneys (Fig. 3), lungs (Fig. 4), heart (Fig. 5) and brain (Fig. 6) revealed PPD toxicity causing severe cellular damage in the PPD-only control group compared to the groups given different supplements (Table 4). The rats treated with olive oil showed a comparable morphology of kidneys, lungs, heart and brain to that of the control negative group. Similarly, the rats treated with coconut oil showed relative improvement in the cellular morphology of these organs as the PPD-only control group (treated only with 30mg/kg PPD). However, the rats treated with methylene blue showed only a minute improvement in the histo-architecture of the cellular damage (Table 4).

DISCUSSION

When ingested, PPD is absorbed through digestive mucus membranes and oxidized into the toxic product known as Bondrowski Base (BB). BB is much more toxic than PPD and causes inflammation of laryngeal cells and rhabdomyolysis, leading to severe respiratory and renal distress (Gude *et al*., 2012). In severe cases, PPD intoxication leads to morbidity and ultimately death of the

Table 4: Effects of supplementation of methylene blue, coconut oil and olive oil on the severity of histo-pathological lesions on visceral organs of albino rats exposed to PPD.

Histo-pathological lesions	Groups / Treatments				
	A	B	C	D	E
Kidneys					
Congestion	-	++++	+++	+++	+++
Increased Bowman's space	-	+++	+++	+++	++
Ceroid formation	-	++++	+++	++	++
Edema	-	++++	++++	+++	+++
Necrosis of tubular cells	-	++++	+++	++	++
Nuclear hypertrophy	-	++++	+++	++	++
Deterioration of glomeruli	-	++++	+++	+++	+++
Degeneration and obliteration of renal tubules	-	++++	+++	+++	+++
Liver					
Congestion	-	++++	+++	+++	+++
Ceroid formation	-	++++	+++	++	++
Vacuolar degeneration	-	++++	++++	+++	+++
Hemorrhages	-	++++	++++	++	++
Pyknosis	-	++++	+++	+++	+++
Nuclear hypertrophy	-	++++	++++	++++	+++
Karyorrhexis	-	++++	++++	++	++
Karyolysis	-	++++	+++	+++	+++
Degeneration of hepatocytes	-	++++	++++	+++	+++
Hepatocytes with eccentric nuclei	-	++++	+++	+++	+++
Lungs					
Congestion	-	++++	+++	+++	+++
Atelectasis	-	++++	++	++	++
Interstitial pneumonia	-	++++	+++	+++	+++
Emphysema	-	++++	+++	+++	++
Inflammatory materials	-	++++	++++	++	++
Edema	-	++++	+++	++	++
Hemorrhages	-	++++	+++	+++	++
Heart					
Myocarditis	-	++++	+++	++	++
Congestion	-	++++	+++	+++	++
Edema	-	++++	++	+++	++
Inflammatory material	-	++++	+++	++	++
Myofibrillosis	-	++++	+++	+++	++
Hemorrhages	-	++++	+++	++	+++
Brain					
Necrosis of neurons	-	++++	+++	++	++
Cytoplasmic vacuolization	-	+++	+++	+++	++
Inter-cellular edema	-	++++	+++	++	++
Congestion of neural cells	-	++++	+++	+++	++

Group A: control negative, Group B: PPD-only control, Group C: PPD injection and supplemented with methylene blue, Group D: PPD injection and supplemented with coconut oil, Group E: PPD injection and supplemented with olive oil

individual occurs in acute cases. The purpose of the current study was to explore an appropriate anti-dote against PPD toxicity.

Blood profile is the best biomarker present in our body and if an injury occurs in a particular organ, the alterations in the number of respective enzymes and factors are depicted in the serum (Tahir *et al*., 2021; Vo *et al*., 2022). The increased level of urea and creatinine depicts renal injury in the animals treated with PPD (Naqvi *et al*., 2015). PPD toxicity causes rhabdomyolysis that leads to the failure of kidneys and ultimately death of the individual (Naqvi *et al*., 2015; Gupta *et al*., 2015). In the case of hepatic and cardiac injury, the levels of specific enzymes such as AST, ALT, Bilirubin and CK-MB are altered in the serum (El-Sarnagawy *et al*., 2023). Methylene blue has significantly decreased the number of renal markers, hence, it seems to have protective effects on the renal tissues against injury, as its effects on kidneys have also been described previously in rats (Usefzay *et al*., 2022) and humans (Samoylova *et al*., 2023).

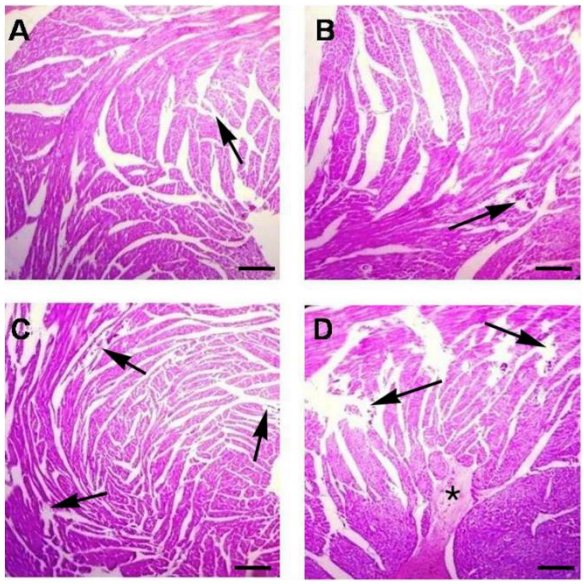

Fig. 5: Photomicrograph of the effects of supplementation of methylene blue, coconut oil and olive oil on histo-pathological lesions in the heart of albino rats exposed to PPD. The presence of inflammatory materials (*) and disruption and inflammation of cardiac myocytes (myocarditis = arrows) was decreased in the groups treated with coconut and olive oils compared to the PPD-only control group. While presence of blood in the interstitial spaces of cardiac myocytes was found to be reduced in the groups treated with different supplements compared to the PPD control group. A: PPD-only control, B: PPD injection and treated with methylene blue, C: PPD injection and treated with coconut oil, D: PPD injection and treated with olive oil. Scale bar = 50µm, 400X, Stain: Hematoxylin and Eosin

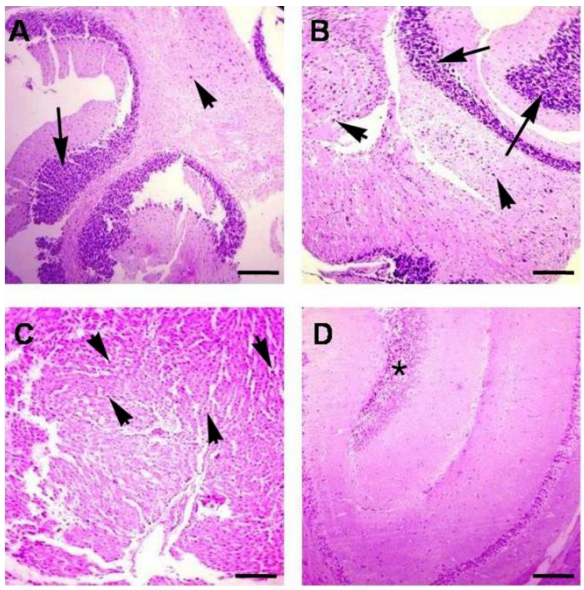

Fig. 6: Photomicrograph of the effects of supplementation of methylene blue, coconut oil and olive oil on histo-pathological lesions in the brain of albino rats exposed to PPD. A)necrosed cells (arrow head) and microgliosis (arrow); B)necrosis and atrophy of neurons (arrow heads) and microgliosis (arrows); C) atrophy and eccentric neuron (arrow heads) and D) microgliosis (*) was found to be relatively decreased in the groups supplemented with coconut and olive oils. A: PPD-only control, B: PPD injection and treated with methylene blue, C: PPD injection and treated with coconut oil, D: PPD injection and treated with olive oil. Scale bar = 100µm, 400X, Stain: Hematoxylin and Eosin.

In the current study, we have observed that coconut and olive oils have a role in decreasing the level of renal markers in PPD toxicity. It has also been previously found that coconut water has protective effects on renal function

and prevents the development of oxidative stress in the kidneys (Kunle-Alabi *et al*., 2019; Gautama *et al*., 2021). It inhibits the deposition of crystals in renal tissue and thus decreases their release in urine (Gandhi *et al*., 2013). The effects of olive oil on urea ($p<0.001$) and creatinine ($p<0.01$) were found to be significant compared to the other two treatments to counter the effects of PPD toxicity. Similarly, olive oil has previously been found to ameliorate the toxicological effects of arsenic trioxide on the hemato-biochemical profile in rabbits (Zubair *et al*., 2022). We have observed a markedly increased level of hepatic markers (AST, ALT and Bilirubin) in the rats of the PPD-only control group treated with PPD. This high level of liver enzymes is depictive of hepatic injury that leads to their increased concentration in the blood (Agrawal *et al*., 2016; Ismail, 2022). While the level of bilirubin is increased in blood due to necrosis of hepatocytes (Ruiz *et al*., 2021). The post-PPD treatment of rats with olive oil significantly decreased the level of these hepatic markers compared to the animals not treated with prophylactic therapy. Olive oil administration significantly improved the oxidative stress biomarkers (MDA, FRAP, GSH) and enzymatic markers (AST, ALT, ALP) of liver injury in rats having sodium arsenate-induced hepato-toxicity (Mohammadian *et al*., 2018). It has also been previously found that methylene blue has protective effects on liver cells after paraquat-induced hepato-toxicity in rats (Zeinvand-Lorestani *et al*., 2018). Similarly, olive oil improved the gross and microscopic histo-pathological alterations induced by arsenic in the hepatic lobes of Albino rats (Cheema and Ali, 2018) and was found to have protective effects in histo-pathological alterations in kidneys and significantly enhanced the anti-oxidant enzymes in male albino rats against ethephon-induced toxicity (Mokhtari *et al*., 2019).

They found high values of cardiac enzymes in rats intoxicated with PPD that are due to cardiac injury that increases the level of CK-MB in the serum (Palanisamy *et al*., 2013). The animals treated with olive oil have significantly decreased levels of CK-MB enzyme, so it seems to have protective effects on the heart from tissue injury. While the other two treatments of animals with coconut oil and methylene blue showed relatively high values of CK-MB like that in the animals of the PPD-only control group shows that these treatments might not have promising protective effects on cardiac tissue against the injury. Due to the production of free radicals, it has been reported that the organs that need sufficient oxygen like the heart, brain, kidneys, lungs and liver are more sensitive to damage by the toxins and that is reflected by histo-pathological changes (Hosseini *et al*., 2020). In the current study, we found that olive oil imparts beneficial effects on renal tissue after PPD injection since the levels of urea and creatinine were like those of control negative animals. Extra virgin olive oil ameliorated the histological changes induced by arsenic and significantly prevented the hypertrophy of epithelial cells of proximal convoluted tubules in both kidneys of albino rats (Haq *et al*., 2023). Olive oil has great health benefits as it prevents coronary heart disease and modifies immune responses.Moreover, it has anti-apoptotic, anti-inflammatory and anti-oxidative properties (Haq *et al*., 2023). Hematological and biochemical parameters result also support the protective effects of methylene blue. So, methylene blue has protective potential against PPD toxicity.

Conclusions: The rats supplemented with methylene blue, coconut and olive oils after PPD exposure showed improvement in the physiological and biochemical parameters. Their health is greatly improved compared to the animals intoxicated with PPD. Hemato-biochemical profile, hepatic and renal markers and CK-MB have been greatly recovered along with histo-pathological lesions on the heart, kidneys, lungs and brain revealed after PPD toxicity be partially reversed by our selective treatments. The animals supplemented with olive oil showed better results than methylene blue and coconut oil supplementations, thus, it could be concluded that olive oil could be used as a possible antidote during the supportive therapy against PPD toxicity.

Conflict of interest: The authors declare no conflict of interest.

Authors contributions: Conceived and designed the project: AZ, SA, RH; Executed the experiment; AZ, SH, MZ, JY; Analyzed the samples: AZ, SA, HMA, RH; Analyzed the data: SA, HMA, RM, MAZ, MAK, AA; All authors critically revised the manuscript for important intellectual contents and approved the final version for publication.

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