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Mitigating ethion-induced hemotoxicity: The role of mango leaf extract and Quercetin

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Abstract
The current study was carried out to understand ethion-induced hemotoxicity in male wistar albino rats, as well as the effect of *Mangifera indica* lead extract (MLE) and Quercetin alone and in combination given orally for 60 days. Following administration, ethion (7.2 $mgkg^{-1}$) caused a significant increase in granulocyte percentage, lymphocyte count, and WBC count, as well as a remarkable reduction in RBC count, hemoglobin, MCHC, MCH, and MCV levels, which was attributed for oxidative stress, reducing RBC lifespan and the drug's immune reaction in the body by ethion. The increase in RDW in ethion-treated rats was also observed as a result of chronic inflammation. Upon pre-administration of MLE (400 $mgkg^{-1}$) and quercetin (50 $mgkg^{-1}$) demonstrated a protective effect in the ethion-treated group, however the combination of both has shown an significant difference in normalizing the toxic effects.

Keywords: Ethion, hemotoxicity, *Mangifera indica*, quercetin, RBC, WBC

Introduction
Global environmental modeling highlights a concerning reality: over 60% of agricultural land worldwide faces imminent contamination risk from multiple pesticide ingredients. This reveals the pervasive vulnerability of vast agricultural landscapes to this complex issue. Additionally, more than 30% of this endangered land falls into the "high risk" category for pollution. Notably, one-third of these high-risk areas boast remarkable biodiversity, intensifying the ecological significance of the challenge (Tang *et al*., 2021) [30].

Pesticides, including specific ones like DDT, chlordane, and heptachlor, disrupt endocrine and reproductive systems in various organisms (Vos *et al*., 2000) [32], impacting both human and ecosystems (UNEP, 200). The pesticide synthesis in India, began during 1952 with DDT and BHC with a growth of 85,000 MT by mid-90s (Gupta, 2004) [9]. In the Asian landscape, India ranks twelfth globally in pesticide production, contributing about 90,000 tons annually (Khan *et al*., 2010) [13]. Organophosphorus (OP) pesticides, widely used against disease vectors, notably contribute to contemporary environmental pollution (Mostafalou and Abdollahi, 2013) [17]. OP impacts depend on dose, exposure, uptake, and physicochemical properties (Mostafalou, 2013) [17].

Ethion, an OPI has become recognized as a widespread environmental pollutant affecting global ecosystems (Foster *et al*., 2004) [6]. In India, the annual application of 3-4K MT of ethion raises significant health concerns, particularly rustic areas (Kalam & Mukerjee, 2001) [11], leading to notable severity and death (Dewan *et al*., 2002) [2]. The compound directly harms vital organs, including the brain, intestine, kidney and liver (Mosha & Gyrd-Hansen, 1990) [16], with acute exposure impacting the nervous system via acetylcholinesterase inhibition (Nath and Kumar, 1999) [20]. Oxidative stress, a key non-cholinergic mechanism linked to organophosphates, is implicated in health issues from pesticide exposure (Soltaninejad and Abdollahi, 2009) [27], although conclusive proof of its role in ethion's chronic toxicity is limited. High morbidity and mortality persist due to ethion exposure, underscoring its serious health implications (Dewan *et al*., 2008) [2], alongside direct harm to organs (Mosha and Gyrd-Hansen, 1990) [16].

Mangifera indica L., commonly known as the mango fruit, originates from tropical Asia (Nwinuka *et al*, 2008 & Ross., 1999) [21, 25]. It's a sizeable tree, reaching 15 to 30 meters in height, with reddish or yellowish leaves measuring around 29 to 30 centimeters, and small red or yellowish-green flowers (Gill 1992, Perpetuo and Salgado 2003; Ojewole 2005; Muruganandan *et al*., 2005; Madunagu *et al*., 1990) [8, 23, 22, 18, 15].

Beyond its fruit, various parts of this tree, including stem bark, roots, and leaves, hold medicinal value for conditions like malaria, diabetes, asthma, and anemia (Ojewole, 2005 & Muruganandan *et al*., 2005) [22, 18].

Quercetin, a widespread flavonoid in plants, exhibits diverse pharmacological effects, including anticancer, antiviral, anti-inflammatory, and cardiovascular benefits. These properties position flavonoids like quercetin as potential therapies for conditions such as cancer, viral infections, allergies, hypertension, and atherosclerosis (Formica and Regelson, 1995; Yoshihisa and Masanori, 2000) [5, 34].

Materials and Methods

Table 1: Experimental protocol

Sr. No	Group	Treatment done (period of exposure is 60 days)
1	I	DMSO (1 $mlkg^{-1}$)
2	II	MLE (400 $mgkg^{-1}$)
3	III	Quercetin (50 $mgkg^{-1}$)
4	IV	Ethion (7.2 $mgkg^{-1}$)
5	V	Ethion (7.2 $mgkg^{-1}$) + MLE (400 $mgkg^{-1}$)
6	VI	Ethion (7.2 $mgkg^{-1}$) + Quercetin (50 $mgkg^{-1)}$
7	VII	Ethion (7.2 $mgkg^{-1}$) + MLE (400 $mgkg^{-1}$) + Quercetin (50 $mgkg^{-1)}$

LD_{50} - Lethal Dose-50 (Derived data - 2 mg/kg). DMSO = Dimethyl Sulfoxide, MLE = Mango Leaves Extract (Methanolic)

Experimental protocol

Male rats of Wistar albino strains (140–190 gms) were bought from the Laboratory Animal Resource, ICAR-Indian Veterinary Research Insitute. Accommodated in sterile polypropylene cages, water and feed were accessed freely with 12-hour light/dark cycle. All procedures adhered to guidelines and in agreement with IAEC of the institute. Seven groups of six rats each were formed randomly (Table-1). The study employed technical-grade Ethion, purchased from Cheminova India Ltd, Mumbai, India and Quercetin dihydrate (purity >95%) from Sigma Aldrich. Local market-bought *Mangifera indica* leaves were shade-dried, Soxhlet-extracted, and the rotary vacuum evaporator condensed methanolic extract used. Rats received ethion, mangoleaf extract, and quercetin orally in line with Table 1. Following final administration, blood obtained from orbital sinuses 24 hours later and blood parameters assessed via hematological analyser (Urit-3000, China).

Data analysis

Statistics data will be analyzed by use of Prism (v 8.0.1) with ANOVA and Multiple comparison using Tukeys test.

Results

Hematology Parameters

Treatment	DMSO 1$mlkg^{-1}$	MLE 400 $mgkg^{-1}$	Quercetin 50 $mgkg^{-1}$	Ethion 7.2 $mgkg^{-1}$	Ethion + MLE	Ethion + Quercetin	Ethion + MLE+ Quercetin
MCHC (in g/L)	325.4±1.95	324.4±2.92	327.9±2.32	251.5±2.99***	274.6±4.19	305.8±3.98*	317.5±1.29$^{\#\#\#}$
RDW- CV (in %)	14.58±0.42	14.95±0.45	13.98±0.43	14.75±0.52*	14.54±0.54	13.67±0.57	13.98±0.28
RDW-SD (in μm^3)	23.76±0.45	24.88±0.35	24.70±0.38	49.41±0.84***	37.69±0.49	29.30±0.28$^{\#\#}$	24.48±0.35$^{\#\#\#}$
PLT (in $10^3/\mu l$)	389.6±5.12	387.4±6.12	384.7±8.24	484.7±6.64***	513.4±16.6	429.4±13.3$^{\#\#}$	394.5±4.05$^{\#\#\#}$
MPV (in fL)	4.23±0.06	4.31±0.09	4.36±0.04	3.58±0.07	4.09±0.08	4.07±0.07	4.35±0.22
PDW (in %)	9.95±0.17	9.87±0.27	10.3±0.17	11.2±0.26*	10.4±0.32	10.58±0.54	11.37±0.36
PCT (in %)	0.42±0.04	0.45±0.04	0.43±0.04	0.45±0.05	0.42±0.02	0.41±0.02	0.48±0.04
P-LCC (in $10^3/\mu l$)	257.4±3.70	259.0±5.27	255.5±3.37	289.4±1.90**	282.8±1.87	274.2±2.99$^{\#}$	262.5±0.65$^{\#\#}$
P-LCR (in %)	35.68±0.42	36.56±0.54	35.54±0.42	36.54±0.75	36.45±0.75	37.75±0.57	38.52±0.32
WBC (in $10^3/\mu l$)	10.89±0.52	10.98±0.57	10.75±0.36	21.47±0.57***	20.94±0.34	18.17±0.45	11.75±0.38$^{\#\#\#}$
Lymphocyte (in $10^3/\mu l$)	7.57±0.66	7.65±0.61	7.59±0.28	19.75±0.47***	18.36±0.26	16.30±0.28	11.57±0.18$^{\#\#\#}$
Mid Cells (in $10^3/\mu l$)	0.24±0.008	0.31±0.017	0.25±0.006	0.79±0.014	0.71±0.011	0.60±0.017	0.40±0.019
Granulocyte (in $10^3/\mu l$)	2.58±0.17	2.61±0.17	2.62±0.17	7.85±0.41***	7.96±0.30	7.79±0.14	3.28±0.24$^{\#\#\#}$
RBC (in $10^6/\mu l$)	6.98±0.75	6.89±0.18	6.75±0.24	5.23±0.08***	5.21±0.08	5.22±0.08	6.65±0.13$^{\#\#\#}$
Hemoglobin (in g/dl)	13.39±0.25	12.39±0.25	12.45±0.26	8.94±0.18***	8.97±0.17	10.74±0.28	12.30±0.18$^{\#\#}$
Hematocrit (in %)	38.51±0.16	38.95±0.17	39.03±0.30	22.85±0.63**	22.91±0.71	28.47±0.37$^{\#}$	36.01±0.42$^{\#\#}$
MCV (in fL)	64.07±0.41	64.57±0.46	63.69±0.33	51.22±0.80**	51.40±0.61	51.70±0.73	63.02±0.45$^{\#\#}$
MCH (in pg)	21.70±0.57	21.36±0.21	21.68±0.25	10.70±0.31***	14.49±0.50	17.29±0.48	20.04±0.13$^{\#\#}$

MLE-Mango Leaves Extract (Methanolic), LD 50 – Lethal Dose-50, MCHC – Mean Corpuscular Hb Concentration, RDW-CV –Redcell distribute width – Coefficint of Variation, RDW-SD – Redcell distibution width – Standrd deviation, PLT – Platelet distribution, MPV –Mean Platelets Volum, PDW – Platelet distribution width, PCT – Procalcitonin Test, P-LCC – Platelet Large Cell Count, P-LCR – Platelet Large Cell Ratio, WBC- White Blood Cells, RBC – Red Blood Cells, MCV –Mean Corpuscular Volume, MCH- Mean Corpuscular Hemoglobin,

Values are expressed in Mean ±SEM, * shown for control group comparison, # shown for Ethion group comparison. The number of superscripts represents the level of significance, $P<0.033$ for one, $P<0.002$ for two and $P< 0.001$ three superscripts.

Discussion

Organophosphate pesticides (OP pesticides) constitute a significant category of chemicals widely utilized in agricultural pest management. These substances find extensive use on a global scale. However, instances of toxicity stemming from these compounds, especially in developing countries like India, pose a substantial public health concern (Shetty., 2004) [26].

Animals exposed to ethion for 60 days showed a significant increased lymphocyte count, granulocyte percentage and WBC count, whereas the values of RBC, hemoglobin, MCHC, MCV and MCH showed a notable decreased in group

exposed to ethion than with control group (Garcia *et al*., 2016) [7]. The increased WBC might be of immune response (Naik *et al*., 2018) [19], the decreased in RBC, hemoglobin, MCV, MCH and MCHC (Rahman *et al*., 1990) [24] caused an anaemic condition due to reduced RBC and hemoglobin (Kumar., 1990) [14]. A decreased Hb levels due to either increased rate of destruction of the hemoglobin or decreased in the hemoglobin synthesis (Kartheek and David, 2017) [12] might be causing hemolysis and reduced RBCs (Jung-Hoon, 2005) [10]. As a result, upon exposure of MLE in combination with the quercetin has significantly highly improved the conditions of animal normalizing both RBCs and Hb levels to the control groups (Abidakun *et al*., 2018) [1].

A considerable rise in counts of basophils and monocytes were observed related to chronic inflammation produced by ethion after sixty days of exposure. Thrombocytosis is caused by an elevated platelet synthesis and damage due to oxidation, which indicates the gravity of exposure (El Sadek *et al*, 1999; Fareed *et al*, 2013; Tang *et al*., 2018; Wafa *et al*., 2013) [3, 4, 29, 33]. Damage by oxidation caused by pesticides, which shortens the red blood cells life preventing its maturation phase (Soltaninejad *et al*, 2009; Garcia *et al*., 2016) [28, 7]. Several studies reported increased redcell width acts as an indicator of inflammation of chronic stage and stress of oxidation (Soltninejad *et al*., 2009) [28].

A remarkable elevation of basophils and monocytes indicates the inflammation of chronic phase upon administration of ethion for sub chronic period. Thrombocytosis occurrence is mainly by virtue of elevated platelets and stress in the cells due to oxidation (Wafa *et al*, 2013, Tang *et al*, 2018, El-Sadek *et al*, 1999, Fareed *et al*, 2013) [33, 29, 3, 4]. The exposure of pesticides causes stress due to oxidative damage and reduces the red blood cells live avoiding the process of maturatin and increased its width, and also an indicator of inflammation of longer time and stress of free radicals (Garcia *et al*., 2016, Soltaninejad *et al*., 2009) [7, 28].

Conclusion

A sub-chronic study of sixty-days of ethion treatment Wistar rats revealed a disordered blood parameters at high dose. Furthermore, co-administration of methanolic mango leaf extract and quercetin and ethion considerably declined the potential of causing the impact of disorded blood parameters, which was greater in the combination of MLE and quercetin than in the individual. While more researches needed with identified primary specific organ toxicosis of ethion, the result of the present study is an indicator of its effect in both inflammation and stress due to release of superoxide molecules.

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