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Insilco prediction and hematological alterations in male rats exposed to Ethion and its amelioration by nano-quercetin: A sub chronic study

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Abstract
Insecticides are frequently used pesticides throughout the world which seem to be indispensable in modern agricultural production systems. In the present study we predicted the in silico ADME (Absorption, Distribution, Metabolism and Elimination) properties and multifarious kinds of biological activity including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression, etc. of the pesticide Ethion, by using SwissADME and PASS Online web portals. *In vivo* hematological alterations following sub-chronic exposure to ethion in male Wistar albino rats and its amelioration by quercetin and nano-quercetin was studied. Ethion cleared all SwissADME filters for the properties like physicochemical property, lipophilicity, water solubility, pharmacokinetics, drug likedness and medicinal chemistry. PASS Online predicted multitudinal adverse effects of ethion, among which, reproductive toxicity predominated. Ethion induced a significant dose dependent increase in hematological parameters like WBC count, lymphocytes and granulocytes percentage, RDW-SD and P-LCC, whereas a decrease was evident in RBC count, MCV, MCH, MCHC, PLT, hemoglobin and hematocrit values as compared to control group respectively. The ethion exposed rats that were co-administered/concurrently dosed with quercetin as well as nano-quercetin evinced a significant ameliorating potential for quercetin as compared to ethion high dose and control.

Keywords: Indian landraces of rice, target production environment, genetic variability, principle component analysis, correlation

Introduction
Pesticides are the broad group chemicals that includes herbicides, insecticides, fungicides, and rodenticides, nematicides of either natural or synthetic origin. These are extensively employed in latitudinal agricultural practices to restrict pest, weeds and diseases in agricultural and horticultural crops. (Sharma *et al.*, 2019) [1]. As per the reports from leading online data repository Statista Research Department, 2022, the global consumption of agricultural pesticides grew most steadily from 1990 to 2020, with pesticide consumption worldwide standing at nearly 2.7 million metric tons, which was increased more than 57 percent as compared to pesticide use for the year 1990 (Statista, 2022) [2]. At global level, in 2020 use of pesticides in agriculture remained stable with consumption of 2.7 million tons (Mt) of active ingredients. The global average for application of pesticides (quantity/area) is 1.8 kg/ha, which transforms to an average of 0.37 kg/person on per capita basis and 0.69 kg/1000 International Dollar (I$) per year in per value of agricultural production/produce basis. Total pesticides trade reached approximately 7.2 Mt of formulated products in 2020, with a value of USD 41.1 billion (FAO, 2022) [4].

Pesticide use in Asia remains below the world average on a per capita, per value of agricultural production and per hectare basis over the whole period of 1990-2022, averaging 0.17 kg per person per year, 0.47 kg per 1000 I$ per year and 1.17 kg per ha per year, respectively. The total use of pesticides in India transforms to 0.37 kg/ha, 0.04 kg/person and 0.14 kg/1000 I$/year in terms of consumption per unit area, per head and per value of annual agricultural production respectively. (FAOSTAT-Pesticide indicators, 2022) [4].

More than half of the pesticides used in India are insecticides (Nayak and Solanki, 2021) [5]. Pesticide use is associated with many ill effects such as residue in plant parts, resistance to insecticides, secondary pest out-break, pollution to natural resources, health complications for human and wildlife etc., which are even more complicated by their excess indiscriminate use.

This has created arguments favoring need for tight regulating their use and switch over to eco-friendly pest management methods (Birthal and Sharma, 2004) [6].

Ethion, is an organophosphate pesticide registered and approved by Government of India under section 9(3) of the Insecticides Act, 1968 at serial number 111 as on July 01st 2022 (GOI, 2022) [7]. It is a small and lipophilic molecule absorbed into organs by passive diffusion. It is primarily metabolized by liver and converted into its active form named ethion monoxon, a potent inhibitor of cholinesterase enzyme (ATSDR, 2022) [8]. Approximately 3000-4000 metric tons of ethion used annually in India which potentially carries with it a major public health hazard especially in rural India (Kalam and Mukerjee, 2001) [9]. Exposure to ethion is associated with high morbidity and mortality (Dewan *et al.*, 2008) [10]. Ethion exposure has been observed to have direct toxic effects on liver, kidney, intestine and brain (Mosha and Gyrd-Hansen. 1990) [11]. The biochemical mechanisms involved in toxicity following chronic low dose exposure to ethion are not fully understood as it does not involve cholinergic mechanisms. Oxidative stress caused by organophosphates is proposed to be a major non-cholinergic mechanism linking pesticide exposure to its health effects (Soltaninejad, K. and Abdollahi, 2009) [65]. Oxidative stress has been reported as one of the mechanisms of toxicity of Ethion via the enhancement of oxidative stress markers and the disruption of antioxidant balance with a resultant over accumulation of reactive oxygen species (ROS) (Banerjee *et al.*, 2001) [13].

Used since the year 1857, Quercetin is a flavonoid having the molecular formula $C_{15}H_{10}O_7$. It is widely found in fruits and vegetables. It has unique biological properties, including potential to improve physical and mental status, and reduce viral infection (Li *et al.*, 2016 [14] and Yang *et al.*, 2020) [15]. The antioxidant and anti-inflammatory properties of quercetin are closely related to their potential use in the prevention and treatment of pesticide and heavy metal poisoning. In addition, quercetin plays an essential role in reducing mycotoxins and protecting cells from damage due to exposure to them (Yang *et al.*, 2020) [15].

Therefore, the present work aimed at

- Elucidating Toxicokinetic, toxicodynamics and target system toxic property of Ethion using In-silico toxicity prediction tools SWISS ADME and PASS Online web servers
- Finding out In-vivo hematological alterations in male Wistar albino rats following sub chronic exposure to Ethion and
- Evaluation of protective potential of quercetin and nano quercetin following sub chronic Ethion exposure.

Materials and Methods

Web database

SwissADME (http://www.swissadme.ch/) and Way2Drug – PASS Online (http://www.way2drug.com/passonline/) Predictive services library were utilized on online platform for in-silico predictions and Pub Chem (https://pubchem.ncbi.nlm.nih.gov/) was used for analysis of chemical structure of Ethion. Swiss ADME (http://www.swissadme.ch/) allows to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, drug-likedness and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery. PASS Online (http://www.way2drug.com/passonline/) predicts over 4000 kinds of biological activity, including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression, etc.

In-vivo Experimental Design, Chemicals and Instruments

Adult male Wistar albino rats weighing 120–180 g were procured from Laboratory Animal Resource Station, IVRI. All the animals were housed in clean polypropylene cages and were fed standard diet ad libitum with free access to water on a 12 h light/dark cycle. All the experiments were performed according to guidelines for use and care of laboratory animals and were approved by the ethical committee of Institutional Animal Ethical Committee (IAEC), IVRI, Izatnagar (IAEC No – 26-1/2022-23/JD(R)/ IAEC. Dated 30.07.2022). The body weight of all the animals was checked regularly and were randomly divided into seven groups, each comprising of six animals (Table-1). Technical grade Ethion, generously gifted by Cheminova India Ltd, Mumbai was used in present study. Quercetin dihydrate (purity above 95%) of Sigma Aldrich GmbH was used in present study. Nano formulation of quercetin was prepared using chitosan and TPP as per method described. Arachis oil was used as a vehicle for oral administration of ethion, quercetin and nano quercetin. Ethion, quercetin and nanoquercetin were administered orally to rats as mentioned in Table 1. The animals were bled from orbital sinus 24 hours after last dose and hematological parameters were determined using Blood Hematology Analyzer (Urit 3000 Vetplus, China).

Statistical analysis

Statistical analysis was done by using GraphPad Prism V 8.0.2 software by applying One way ANOVA followed by Tukeys's multiple comparison test.

Table 1: Experimental grouping of animals

Sl. No	Groups	Treatment	Dose / route (Oral)	Number of Rats	Period of Exposure
1	I	Arachis oil Control	1 ml/kg	6	90 days
2	II	Quercetin Control	50 mg /kg	6	90 days
3	III	Quercetin Nanoparticle Control	50 mg/kg	6	90 days
4	IV	Ethion (1/10th LD 50)	7.2 mg/kg	6	90 days
5	V	Ethion (1/20th LD 50)	3.6 mg/kg	6	90 days
6	VI	Ethion (1/10th LD50) + Quercetin	II + IV	6	90 days
7	VII	Ethion (1/10th LD50) + Nanoquercetin	III + IV	6	90 days

LD 50- Lethal Dose 50 (72 mg/kg)

Results

Computed Descriptors

- Chemical / IUPAC Name- O,O,O′,O′-Tetraethyl S,S′-methylene bis (phosphorodithioate)
- Trade name of ethion includes Bladan®, Rodicide®, and Nialate®
- Depositor supplied synonyms – Diethion, Ethanox, Ethopaz, Nialate, Embathion, Rhodocide, Ethodan, 563-12-2, RP 8167, FMC 1240 and NIA 1240.
- Molecular Formula - $C_9H_{22}O_4P_2S_4$

- Molecular Weight - 384.5
- In ChI - 1S/C9H22O4P2S4/c1-5-10-14(16,11-6-2)18-9-19-15(17,12-7-3)13-8-4/h5-9H2,1-4H3
- Canonical SMILES – CCOP(=S)(OCC)SCSP(=S)(OCC)OCC
- CAS - 563-12-2
- European Community (EC) Number - 209-242-3
- ICSC Number – 0888
- DSS Tox Substance ID - DTXSID2024086

Table 1: Physicochemical Properties

Formula	C9H22O4P2S4
Molecular weight	384.48 g/mol
Num. heavy atoms	19
Num. arom. heavy atoms	0
Fraction Csp3	1.00
Num. rotatable bonds	12
Num. H-bond acceptors	4
Num. H-bond donors	0
Molar Refractivity	96.03
TPSA	171.32 Å²

Table 2: Water Solubility

Log *S* (ESOL)	-4.63
Solubility	9.10e-03 mg/ml; 2.37e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-8.41
Solubility	1.50e-06 mg/ml; 3.89e-09 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-3.01
Solubility	3.72e-01 mg/ml; 9.68e-04 mol/l
Class	Soluble

Table 3: Lipophilicity

Log $P_{o/w}$ (iLOGP)	3.52
Log $P_{o/w}$ (XLOGP3)	5.07
Log $P_{o/w}$ (WLOGP)	6.31
Log $P_{o/w}$ (MLOGP)	0.44
Log $P_{o/w}$ (SILICOS-IT)	5.01
Consensus Log $P_{o/w}$	4.07

Table 4: Pharmacokinetics

GI absorption	Low
BBB Permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP1A2 inhibitor	Yes
CYP1A2 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.05 cm/s

Table 5: Drug-likeness

Lipinski	Yes; 0 violation
Ghose	No; 1 violation: WLOGP>5.6
Veber	No; 2 violations: Rotors>10, TPSA>140
Egan	No; 2 violations: WLOGP>5.88, TPSA>131.6
Muegge	No; 2 violations: XLOGP3>5, TPSA>150
Bioavailability Score	0.55

Table 6: Medicinal Chemistry

PAINS	0 alert
Brenk	2 alerts: het-C-het_not_in_ring, phosphor
Lead-likeness	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5
Synthetic accessibility	4.55

Table 7: PASS: Predicting the Activity Spectra of biologically active Substances of Ethion

Pa – Probability to be active, Pi – Probability to be inactive, Pa>0,7								
Pa	**Pi**	**Activity**	**Pa**	**Pi**	**Activity**	**Pa**	**Pi**	**Activity**
0,976	0,002	Flavin-containing substrate	0,949	0,003	CYP2C19 substrate	0,870	0,008	CYP3A4 substrate
0,965	0,003	CYP2B substrate	0,948	0,003	CYP2D substrate	0,865	0,004	Cutinase inhibitor
0,963	0,001	CYP2C18 substrate	0,944	0,003	CYP2D6 substrate	0,849	0,004	Acetyl esterase inhibitor
0,960	0,003	CYP1A substrate	0,940	0,003	CYP2B6 substrate	0,854	0,009	CYP3A substrate
0,954	0,003	CYP2C9 substrate	0,924	0,001	FMO1 substrate	0,823	0,001	Insecticide
0,951	0,004	CYP2C substrate	0,893	0,001	Acaricide	0,843	0,022	Aspulvinone dimethyl allyl transferase inhibitor
0,950	0,003	CYP1A1 substrate	0,894	0,004	Skin irritation, inactive	0,823	0,003	Antiparasitic
0,814	0,014	Sugar-phosphatase inhibitor	0,784	0,010	5-O-(4-coumaroyl)-D-quinate MAO inhibtion	0,776	0,010	Respiratory analeptic
0,770	0,004	Eye irritation, inactive	0,800	0,036	CYP2C12 substrate	0,764	0,009	2-Hydroxymuconate hydrolase inhibitor
0,752	0,017	TP53 expression enhancer	0,736	0,002	Pediculicide	0,725	0,004	Aryldialkylphosphatase inhibitor
0,717	0,018	Ribulose-phosphate 3-epimerase inhibitor	0,717	0,024	Glutamyl endopeptidase II inhibitor	0,702	0,009	Lysostaphin inhibitor

Table 8: PASS: Predicting the Toxicity Spectra of biologically active Substances of Ethion Possible adverse effects (Pa>0, 7)

Pa	**Pi**	**Activity**	**Pa**	**Pi**	**Activity**	**Pa**	**Pi**	**Activity**
0,994	0,003	Drowsiness	0,989	0,004	Sleep disturbance	0,934	0,005	Dyskinesia
0,939	0,013	Toxic, reproduction	0,921	0,005	Anemia	0,906	0,005	Ataxia
0,889	0,008	Hypotension	0,893	0,012	Hematotoxic	0,875	0,005	Non-mutagenic, Salmonella
0,876	0,014	Toxic, gastrointestinal	0,866	0,008	Dyspnea	0,857	0,007	Tremor
0,851	0,008	Coma	0,845	0,020	Toxic	0,835	0,019	Behavioral disturbance
0,814	0,003	Lacrimal secretion stimulant	0,822	0,014	Respiratory failure	0,785	0,005	Eye irritation, high
0,793	0,018	Neurotoxic	0,794	0,022	Ocular toxicity	0,791	0,019	Toxic, vascular
0,775	0,004	Skin irritation, weak	0,771	0,004	Miotic	0,770	0,005	Eye irritation, moderate
0,772	0,013	Hyperglycemic	0,753	0,005	Skin irritative effect	0,773	0,028	Nausea
0,746	0,004	Eye irritation, weak	0,757	0,021	Consciousness alteration	0,729	0,007	Carcinogenic
0,723	0,004	Mutagenic	0,737	0,019	Embryotoxic	0,731	0,019	Teratogen

Table 9: Hematology Parameters

Treatment	Control (Arachis Oil)	Control (Quercetin) 50 m/kg	Control (NQ) 50 mg/kg	Ethion (1/10 LD50) 7.2 mg/kg	Ethion (1/20 LD50) 3.6 mg/kg	Ethion (1/10 LD50) + Quercetin	Ethion (1/10 LD50) + NQ
MCHC (g/L)	315.4±2.85	316.4±3.82	314.9±3.12	261.3±3.79***	287.4±5.08*	301.7±4.18*	318.1±1.09###
RDW- CV (%)	13.34±0.35	13.85±0.33	12.38±0.23	15.37±0.50*	13.23±0.15	14.53±0.58	14.91±0.08
RDW-SD (μm^3)	24.86±0.34	26.58±0.25	25.90±0.28	48.44±0.94***	37.69±0.49*	28.30±0.28##	25.58±0.35###
PLT ($10^3/\mu l$)	399.9±6.02	390.3±7.78	395.6±8.19	491.6±6.97***	503.3±18.8*	432.3±14.4##	392.1±3.16###
MPV (fL)	4.13±0.05	4.21±0.08	4.26±0.07	3.48±0.04	4.08±0.04	4.06±0.05	4.33±0.11
PDW (%)	9.90±0.19	9.86±0.26	10.2±0.16	11.7±0.28*	10.9±0.23*	10.95±0.25	11.38±0.32
PCT (%)	0.40±0.04	0.40±0.04	0.40±0.04	0.35±0.05	0.43±0.02	0.45±0.02	0.48±0.04
P-LCC ($10^3/\mu l$)	251.4±3.70	257.0±5.27	253.5±3.37	277.4±1.80**	272.8±1.59*	271.2±2.99#	261.5±0.65##
P-LCR (%)	36.96±0.41	37.64±0.28	36.67±0.44	39.62±0.84	37.24±0.67	38.42±0.44	40.62±0.23
WBC ($10^3/\mu l$)	11.88±0.32	11.95±0.51	11.66±0.29	20.23±0.54***	12.94±0.30	13.18±0.43	11.75±0.38###
Lymphocyte ($10^3/\mu l$)	7.48±0.66	7.90±0.61	7.51±0.28	18.68±0.44***	16.36±0.26*	14.30±0.27	10.58±0.19###
Mid Cells ($10^3/\mu l$)	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 ($10^3/\mu l$)	2.64±0.16	2.27±0.19	2.34±0.10	7.90±0.45***	5.96±0.30*	4.77±0.14	3.27±0.21###
RBC ($10^6/\mu l$)	6.69±0.29	6.57±0.17	6.67±0.11	5.33±0.07***	5.91±0.09**	5.92±0.09	6.55±0.12###
Hemoglobin (g/dl)	12.39±0.24	12.36±0.23	12.16±0.27	9.94±0.12***	10.57±0.17*	11.74±0.21	12.10±0.16##
Hematocrit (%)	38.51±0.16	38.95±0.17	39.03±0.30	22.85±0.63**	22.91±0.71*	32.47±0.37	36.01±0.42##
MCV(fL)	64.07±0.41	64.57±0.46	63.69±0.33	51.22±0.80**	59.40±0.61	61.70±0.73	63.02±0.45##
MCH(pg)	20.70±0.56	22.26±0.19	20.66±0.36	10.70±0.31***	14.49±0.50	17.29±0.48	19.04±0.12##

NQ- Nano Quercetin, LD 50 – Lethal Dose 50, WBC- White Blood Cells, RBC – Red Blood Cells, MCV –Mean Corpuscular Volume, MCH- Mean Corpuscular Hemoglobin, MCHC – Mean Corpuscular Hemoglobin Concentration, RDW-CV –Red cell distribution width – Coefficient of Variation, RDW-SD – Red cell distribution width – Standard deviation, PLT – Platelets, MPV –Mean Platelet Volume, PDW – Platelet Distribution Width, PCT – Procalcitonin Test, P-LCC – Platelet Large Cell Count, P-LCR – Platelet Large Cell Ratio

*Values (Mean ± SEM, n=6) bearing superscript in single column are significantly different in One way ANOVA followed by Tukeys's multiple comparison test. Comparison with control group (Arachis oil) and 1/10th LD50 group has shown in * and # accordingly. The number of superscript indicates the level of significance $p < 0.033$, $p < 0.002$ and P< 0.001 are represented by one, two and three superscripts respectively.

Discussion and Summary

Despite increases in pesticide use in agricultural sectors over the last the decennium, both in-vivo and in-vitro toxicological experimentations have kept multifarious hazardous pesticides confined for the purpose of commercial, agricultural, household and public health respectively (Fenner, 2001) [17]. The present study was designed to evaluate the in silico kinetic, dynamics and toxicological properties of ethion and the hematological alterations caused by sub chronic exposure (90 days) to ethion in male rats and its amelioration by quercetin and nano-quercetin respectively.

Swiss ADME is a web tool developed by molecular developing group of Swiss institute of Bioinformatics to validate the properties of drugs (Daina *et al.*, 2017) [18] where the descriptors are reported by computing with OpenBabel (O'Boyle *et al.*, 2011 [19], Delaney *et al.*, 2004 [20], Ali *et al.*, 2012 [21], Potts and Guy, 1992) [22]. Lipophilicity is the partition coefficient between n-octanol and water (log $P_{o/w}$) (Pliska *et al.*, 1996 [23], Arnott *et al.*, 2012) [24]. Swiss ADME give assess to five unrecompensed predictive tools i.e. XLOGP3 (atomistic method), WLOGP (fragmental system), MLOGP (topological method), SILICOS-IT (hybrid method rely on fragments and topological descriptors) and iLOGP (Generalized-Born and solvent accessible surface area (GB/SA) model) (Cheng *et al.*, 2007 [25], Wildman & Crippen, 1999 [26], Moriguchi *et al.*, 1992 [27], Moriguchi *et al.*, 1994 [28]). The multilinear regression models presented demonstrates the capacity to linearly correlate experimental log $P_{o/w}$ with solvation free energy computed in implicit media by the GB/SA approach (Daina *et al* 2014) [18]. Swiss ADME estimated the molecular weight of ethion to be 384.48 g/mol, suitable as drug candidate and the range of experimental log $P_{o/w}$ values of ethion is broad and ranges from 0.44-6.31 with the distribution appearing nearly normal and suggesting the compound to be highly lipophilic. Three topological models are presented in Swiss ADME to predict water solubility ESOL model, SILICOS-IT model and Ali model with different seminal general solubility (Delaney *et al.*, 2004 [20], Ali *et al.*, 2012) [21]. The linear correlation between predicted and experimental values for ESOL and Ali models ranges from 0.61-0.85 respectively and 0.75 for SILICOS-IT (Yalkowsky & Valvani, 1980) [29]. The computation values of Ethion in Swiss ADME suggests a poor to moderate water solubility. The superfamily of CYP isoenzymes plays a key role in drug elimination (Testa *et al.*, 2007) [30], five major CYP isoforms includes CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 (Wolf *et al.*, 2000 [31], Di, 2014) [32] for biotransformation and renal clearance of toxic metabolites. Inhibitors of these enzymes leads to pharmacokinetic drug-drug interactions (Hollenberg, 2002, Huang *et al.*, 2008) causing toxicity, and their bioaccumulation (Kirchmair *et al.*, 2015) [35]. Ethion inhibits all isoenzymes forms except CYP2D6. The linear progressive skin permeation depends on the theory of QSPR model by Potts and Guy, expressing the skin permeation coefficient (Log K_p) as decimal logarithm in cm/s respectively. More negative the value corresponds to the lower skin penetration, as ethion showed -5.05 cm/sec, might be categorized to have moderate to good skin penetrating property.

Drug-likeness describes the chance of any compound to be an oral drug with respect to bioavailability. The SwissADME gives passage to five divergent rule based filters, namely Lipinski (Lipinski *et al.*, 2001) [36], Ghose (Ghose *et al.*, 1999) [37], Veber (Veber *et al.*, 2002) [38], Egan (Egan *et al.*, 2000) [39] and Muegge (Muegge *et al.*, 2001) [40]. Upon analysis, ethion has found to be drug-like, following the rules set by all filters with minor violations. The bioavailability score predicts the probability of a molecule to have about 10% oral bioavailability in rats or measurable $CaCo_2$ permeability.

PAINS (Pan Assay INterference compoundS) are the

molecules with fragments reporting positive biological output. It analysis/analyses six orthogonal assays and active molecule break assay with 481 recurrent fragments, which is supposed to be promiscuous compounds by displaying warning for the molecule under evaluation (Baell & Holloway, 2010) [41] and with BRENK analyzing 105 fragments corresponds to putative toxicity of the molecule (Brenk *et al.*, 2008) [42]. Leadlikenss is the molecular entity suitable for optimization. For the molecule under analysis, synthetic accessibility is the sum of fragmental contributions in terms of size and complexity like macrocycles, spiro functions, chiral centers, from which the SA scores ranges from 1 (very easy) to 10 (Very difficult) (Ertl & Schuffenhauer, 2009) [43].

PASS Online (Prediction of activity spectra of substances) is a web based tool which predicts biological activity of even those compounds which are not yet synthesized or tested, based on their chemical structures and predicts their pharmacological and toxicological effects. It also predicts their interaction with enzymes, transporters and influence on gene expression (Basha *et al.*, 2018) [44]. Mean accuracy of prediction of compounds is about 90% which is very much suitable for finding and optimizing new lead compounds (Stepanchikova *et al.*, 2003) [45]. PASS estimates the possibilities of a substance to be active or inactive based on SAR (Structure activity relationship) base containing vocabulary of MNA descriptors (Multilevel neighborhoods of atoms). The result of algorithm of prediction has a list of bioactivity names with pertinent values and the values represent the if a given activity type either revealed (Pa) or not revealed (Pi) in a scale of 0.000 to 1.000 for each activity type from biological activity spectrum. Only those activity types are considered possible for which Pa > Pi (Poroikov *et al.*, 2001). Ethion showed a maximum predictive activity spectra *on flavin containing substrate and* on multifarious CYP isoenzymes, with highest toxicity spectrum exhibited towards disturbance in sleep and a potent reproductive toxicity respectively.

Available information suggests that pesticides can have long-term adverse effects on the reproductive, neurological, respiratory, and hematopoietic systems in humans. Moreover, long-term exposure to pesticides may also cause various types of cancer, including multiple myeloma, leukemia, and non-Hodgkin lymphoma (Gangemi *et al.*, 2016) [49]. Subchronic exposure of rats with Ethion showed a dose dependent and significant increase in values of RBC count, WBC count, and increased lymphocytes and granulocytes percentage in differential count. Mean values of MCH, MCHC, hemoglobin values were significantly decreased in ethion treated group as compared with control group. Lowering of MCHC values is a significant indicator of anaemia (Nejatifar *et al.*, 2022, Garcia *et al.*, 2016, Acker and Nogueira, 2012) [48, 47, 50]. The WBC count has been significantly increased perhaps due to immune response to exposure of pesticides (Garcia *et al.*, 2016 [47], Aradhna Singh *et al.*, 2014 [59], Cortes-Iza *et al.*, 2017) [47, 59, 60]. A significant increase in monocyte and basophil counts corresponds to the chronic inflammation caused by ethion upon exposure for a period of ninety days. The occurrence of thrombocytosis is due to increase in platelet production and oxidative stress indirectly suggesting the level of exposure (Tang *et al.*, 2018, Wafa *et al.*, 2013, Fareed *et al.*, 2013, El-Sadek *et al.*, 1999) [61-64].

Insecticide exposure causes oxidative stress, leads to shorten the lifespan of RBCs by preventing its maturation, several studies reported a high levels of RDW, which act as prognostic marker for chronic inflammation and oxidative stress (Garcia *et al.*, 2016, Soltaninejad *et al.*, 2009) [47, 65].

Quercetin, an flavonoid present in vegetables, onions, green tea, red grape wine, grains and citrus fruits (Amalia *et al.*, 2007 [52], Hou *et al.*, 2014 [53]). It is an oxygen free radical scavenger and a chelator of metal (Behling *et al.*, 2006 [54]; Ozyurt *et al.*, 2014 [55]). Quercetin also protects tissues and cells from the oxidative stress caused by OP pesticides (Kalender *et al.*, 2012 [56]; Uzun & Kalender, 2013, Padma *et al.*, 2012) [56, 57, 58]. In the present study nanoquercetin co-administration in rat group showed significantly lower RBC count, WBC count and increased lymphocytes and granulocytes percentage in differential count as well as the lowering of MCHC values. Similarly Nano quercetin treatment in pesticide challenged rats has been also reported to maintain MCHC levels at near physiological normal values. (Suhas *et al*., 2021) [51].

The effects of ethion on hematological parameters have a significant consequences, and can be considered as an index for chronic inflammation and oxidative stress, the rise in the WBC, lymphocytes, granulocytes and RDW and significant decrease in MCV, MCH, MCHC, hemoglobin, hematocrit and RC values respectively (Afshar *et al.*, 2008, Muthuviveganandavel *et al.*, 2008) [66, 67]. In the present study Quercetin in the form of nano quercetin has significantly normalized the hemotoxic effect of ethion.

Conclusion

The insilico tools plays a quintessential role for predicting the multifarious characteristics of the molecules with respect to pharmacokinetics, pharmacodynamics and toxicity prediction, A comprehensive study was conducted to report the ADME property of ethion by use of Swiss ADME and pharmacodynamics and toxicity prediction by use of PASS Online web server. Ethion has cleared all the filters put forth by Swiss ADME to be called drug-likeness and lead-likeness. Multifactorial dynamic property and toxicity profile evaluated for ethion using PASS Online, priming for reproductive toxicity. A properly conducted sub chronic ninety days exposure of ethion pesticide on male Wistar albino rats reported the hemotoxic effect for high dose i.e. $1/10^{th}$ of LD50. Furthermore, quercetin and nano-quercetin co administration with ethion, significantly reduced the intensity of hemotoxic effect which was more in nano formulation as compared to normal quercetin. Whereof, a detailed research is required to investigate the prime target organ toxicity of ethion, further hemotoxic effects of ethion can be considered as a prima facie for chronic inflammation and oxidative stress marker.

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