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An in vitro study of cytotoxicity of organophosphate insecticides (Imidacloprid, Profenofos, Dichlorvos) and natural products (Neem oil and Dashparni ark) on human peripheral lymphocytes by MTT and Trypan blue assay

Research Article

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Abstract

Human population of India is increasing very fast. Everybody needs food to survive. Agricultural products must be boosted by adding adequate fertilisers and using appropriate insecticides. Organophosphates are one of the most frequently used insecticides. Their overuse leads to the contamination by agricultural runoff. The insecticides may enter drinking water as well. Since organophosphates are acetylcholinesterase inhibitors, they can be dangerous for human health. Hence, a toxicity study by MTT and Trypan Blue Assay of three common insecticides (Imidacloprid, Profenofos, Dichlorvos) and two natural products (*Dashparnik ark* and *Neem* oil) on lymphocytes was taken up. It was found that at 4 hours of incubation at 1mM Imidacloprid showed the greatest drop in viability followed by Dichlorvos and the least harm was caused by Profenofos. For 18 hours of incubation, the same trend was observed, but the decrease and increase were more pronounced. In the case of Profenofos and Dichlorvos the viability percent rises above that of the control. It was probably due to the defense mechanism involving the P450 detoxification pathway of the cells. The damage to the cells was of lesser magnitude when organic insecticides were used. *Neem* nano-drop emulsion showed a significant fall in viability at 2mg/ml. *Dashparnik ark* produced very little damage, but at higher concentration it boosted the viability. Apparently, the extract of leaves fermented in cow urine and cow dung was less damaging than that of other insecticides. Thus, organic insecticides are safer to use because they are ecofriendly and do not harm non-target organisms.

Keywords: Cytotoxicity, Lymphocytes, Insecticides, *Dashparni ark, Neem*.

Introduction

It is well known that the global population is continuously increasing. The global population was 7 billion in 2010 but is expected to reach 9 billion in 2045.(1) The population increase is even steeper in India. Indian population is currently 1.4 billion and is projected to exceed 1.5 billion in 2050.(2) To feed a continuously increasing population, an increase in food grain and crop production is necessary. Intense efforts have to be made to increase production of food grains seasonally. The agricultural sector contributes a major share (22.19%) to the gross value added (GVA) of the GDP of the country (46.4 lakh crore). So, an increase in agricultural production will also give a boost to the economy of our country.

Insecticides have been used for a long time to reduce crop losses, and many types of insecticides have been used in the agricultural industry by almost all types of farmers. The indiscriminate and frequent use of insecticides has resulted in soil and water pollution. This is mainly due to agricultural runoff. If humans are overexposed to these chemicals, they suffer from several ill effects, primarily because most of the insecticides used are organophosphates, which are acetylcholinesterase inhibitors. As such, they hinder nerve impulse transmission, and the target has to suffer the consequences. Therefore, it was thought beneficial to study their toxicity on human peripheral blood lymphocytes in vitro. Some organic insecticides are reportedly less toxic, so in this study, the cytotoxicity of the chemical insecticides has been compared with that of *Neem* oil nano-particle emulsion and *Dashparni Ark*.

Profenofos

Organophosphates are the most widely used group of pesticides globally. Profenofos is a very

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commonly used organophosphate insecticide for fruit and vegetable crops as well as feed crops. According to WHO classification, it is put in the moderately hazardous category (Toxicity class II) and its residues have been found in Okra, Curry leaves, Coriander and Mint. When it is sprayed in fields, it spreads in the air and water also. Dietary intake in humans is common. Many bacterial strains degrade Profenofos, but no complete biodegradation pathway has been discovered for Profenofos. It causes neuro toxicity to vertebrates and macro invertebrates. In humans exposed to Profenofos, chromosomal aberration, necrosis and apoptosis may occur.(3)

Dichlorvos

Dichlorvos, often written as DDVP, is an organophosphate insecticide extensively used to control household pests and also protects stored products from insects. Since it is now known that it is toxic to many more organisms besides insects and its reach is extensive as it causes water pollution, the insecticide has reportedly been banned in European countries since 1998. However, it is used by farmers in India. It is effective against caterpillars, thrips, aphids in horticultural and field crops. Milling & Grain handling industries also use it. It is also used in pet flea collars. Like other organophosphate insecticides, it blocks the enzyme acetylcholinesterase, which inhibits nerve impulse transmission in insects. It is now known that it causes DNA damage in insects.(4)

It causes symptoms like weakness, tightness feeling in chest, nausea, vomiting and even cardiac arrhythmia in humans after prolonged exposure. In humans, some workers have reported that it effects DNA growth and it interferes with the human nervous system.(4) The lethal dose on rats is reported to be 17mg/kg in rat (30-110 mg/kg).(5)

Imidacloprid

Imidacloprid is a systemic, neonicotinoid insecticide. It blocks the nitcotinergic neuronal pathway, so transmission of nerve impulses is blocked in insects resulting in the paralysis and probably the death of the insects. Its binding is very strong in insect neurons but less in mammals, so its toxicity is also less in mammals. It is also used for Termite control apart from pests of crops and also tree injection and protection against boring insects. It is highly soluble in water, so the chance of its being the cause of pollution by runoff water is great, but it undergoes photolysis in water rapidly (half-life: 1-3 yrs). The health risk of Imidacloprid was found to be below 1 as worked by some workers.(6) An analysis of Imidacloprid residues in food showed that of the samples analysed, 2% of the fruit samples, 5.7% of the vegetables samples and 3% of cereal samples showed Imidacloprid residues above the maximal limit. However, they do not pose a health hazard if the amount ingested is calculated according to daily intake.(7) Several pharmacological studies on Imidacloprid have been taken up.Tolerance for imidacloprid residue in food (in eggs) is 0.02ppm.(8)

Neem Oil

Neem

Neem leaves have several therapeutic uses. These are used for their anti-inflammatory and anti-oxidant in traditional medicine.(9) Aqueous *Neem* extract is reported to increase the lymphocyte count in rats when fed for 10 weeks.(10) Its seed oil at a dose of 2ml/kg body weight showed 53.12% inhibition of edema in rats.(11)

Neem Oil

The triglyceride constituents found in the *Neem* seeds are the most extensively studied triterpenoids in *Neem* Oil. The azadirachtin is 300 to over 200 ppm depending on the quality and type of seeds and the method of extraction. Azadirachtin protects crops from pests. It is a well-known fact that chemical insecticides or pesticides which are extensively used for crop protection have a detrimental effect on the environment. As a result, in many western countries the use of many of these has been banned and people are switching to the use of pesticides of biological origin which stop the insects from causing much harm to the crops but at the same time are not very harmful to the environment.

The main constituent of *Neem* Oil is Azadirachtin. It is the most well-known triterpenoid in *Neem* oil. Nimbin is another triterpenoid due to which *Neem* oil has antiseptic, antihistamine and antifungal properties. *Neem* oil also contains several steroids such as stigmasterol, campastrol, etc. Azadirachtin is an ecdysone (the molding hormone of insects) blocker so it interferes with their growth and metamorphosis. It is a feeding deterrent for insects. It is an effective insecticide used against common crop and horticultural pests such as aphids, thrips, beetles, gnats. Insecticidal effects of *Neem* oils were observed in Sudan on pests of stored grains.(12) Bio-pesticidal properties of *Neem* oil was studied by *Agbo et al* 2019.(13)

For the present investigation nano emulsion of *Neem* was used to test its effect on viability of cultured human lymphocytes in vitro as it is an improved drug delivery system. This is due to its greater bioavailability and higher physical stability. The improved bio accessibility is due to the greater surface area and lower surface tension due to nano drops. They can also be used as a substitute for liposomes.

Dashparni Ark, pest and insect repellant

It is an insecticide spray made with plant extracts and fermented in native cow urine and cow dung. As it is a completely natural plant- based product and contains no chemicals, apparently it is completely ecofriendly. For use as a spray, 200ml is mixed in 10 litres of water and the spraying is repeated once in every 10 days or a fortnight. It controls various types of sucking insects such as jassids and aphids. 2kg each of the leaves of *Karanja, Dhatura, Papaya, Tulsi, Marigold, Bael,* Oleander and *Neem* are added to 200 litres of water and mixed. On the second day, 5kg each of crushed Tobacco leaves, Garlic, crushed Turmeric and Chilli, Dry Ginger and 10kg cow urine, 2kg cow dung are added and placed in shade for 40 days with

stirring. This solution can be used after filtration for 6 months and sprayed at 5-8 litre in 200 litre water/acre land.(14)

According to some authors, 2kg each of *Lantana camara (L.), Jatropha curcas (L.) (Ratanjot), Tinospora cordifolia (thumb.) (Giloy)* and castor leaves can also be used and chilli, tobacco, garlic and turmeric can be mixed. 2.5 litre in 200 litre for one acre is used for spray.(15)

It is well known that the leaves of *Tulsi* and *Neem* possess insecticidal properties. Oil from *Karanja* leaves (*Pongamia pinnata (L.))* inhibits the growth of all stages of insects. It serves as a natural pest repellent especially against aphids. *Dhatura* has been found to have fumigant properties. They have been found to be of high value against storage grain pest like beetles. It is especially active against *Callosobronchus*.(16) Tobacco leaf extract is a natural pesticide which inhibits the growth of the insects and kills them also. It is effective against Colorado potato beetle and 11 types of fungi. The concentrated emulsion extract was found to be effective against aphids and other insects of the field. (17) Oleander leaves are also used widely in pest control. The extract contains metabolites of terpenoids which are effective for insect control.

Cow urine is very useful to control the pests in crops and field. It contains traces of metals and non-metals such as zinc, potassium, copper, iron and sulphur. It is a cheap and effective alternative to chemical pesticides and it is especially active against aphids. Besides, it also acts as a urea supplement for plants and improves the insect resistance power of the plants. Cow dung is traditional manure and among its many uses it is a great substitute for pesticides because it contains substances that are rich in antibacterial properties.

The toxic effects of inorganic insecticides on human have been reported by many workers. Hence this study was designed to investigate the *in vitro* cytotoxicity of organophosphate insecticides (Imidacloprid, Profenofos, Dichlorvos) and natural products (*Neem* oil and *Dashparni ark*) on human peripheral lymphocytes using MTT and Trypan blue assay to find an alternate to the inorganic insecticides.

Materials and Methods

Imidacloprid

Chemical Formula: $C_9H_{10}ClN_5O_2$

Molecular weight: 255.66g

Usually for most insects 1L is diluted in 500-700 lit water for spraying. The concentrations of 1mM, 4mM, 8mM and 12mM were taken up for study because the cytotoxicity of various forms of Imdiacloprid to human lymphocytes as checked by Trypan blue assay was found to range from $1.7x10^{-3}$ M to $2.0x10^{-3}$ M.(18)

Profex (Manufactured by: N.A.C.L Industries Limited, Hyderabad, Andhra Pradesh)

It has 40% Profenofos w/w and cypermethrin 4% w/w along with dodecyl benzene sulphonic acid (calcium salt, *Soyabean* oil and solvent in appropriate concentrations to make it is sprayable emulsion).

Structural Formula (Profenofos)

Chemical Formula: $C_{11}H_{15}BrClO_3PS$

Molecular weight: 373.63g

Profenofos is an insecticide of the organophosphate category. It is a pale-yellow liquid with an odour of garlic.

Dichlorvos (Manufactured by Insecticides (India) limited, Alwar, Rajasthan)

Dichlorvos (83.0%) w/w contained xylene, triethanolamine, methylene blue and emulsifier

Chemical Formula: $C_4H_7C_{l2}O_4P$

Molecular Weight: 220.97gm

Preparation of Solutions

The 1mM, 4mM, 8mM and 12mM solutions of Imidacloprid, Dichlorvos and Profenofos were made in distilled water by adding appropriate concentrations depending upon their molecular weight. For instance, 25.5mg of Imidacloprid was dissolved in 10ml distilled water to give 1mM solution (molecular weight: 255.6g), 3.73mg of Profenofos in 10ml distilled water give a 1mM solution (molecular weight: 373.63g), for Dichlorvos (molecular weight: 220.97g), 2.20mg in 10ml give 1mM solution. The 4mM, 8mM and 12mM concentrations were made after adding appropriately calculated amounts in 10ml distilled water.

Neem Oil Nano emulsion

An emulsion of *Neem* oil was made by adding Tween 20 and distilled water in the ratio of 1:3. To 100ml of this was added 100mg of *Neem* oil to give a 1.0 mg/ml emulsion, 120mg of *Neem* oil was added 100ml of tween and water mixture to give 1.2mg/ml emulsion, similarly 150mg, 170mg and 200mg of *Neem* oil were added to give 1.5mg/ml, 17.7mg/ml and 2.0mg/ml emulsion. 100ml of each of these solutions were subjected to ultrasonic vibrations in Rivotek ultrasonicator and sonicated for 30 seconds, the duration of each cycle was 15 seconds. The size of nanoparticles was between 30-70 nm and they are reportedly stable for many months/years.(19)

Dashparni Ark

Dashparni Ark was filtered and used. As it is sprayed @200ml in 10ml of water, solutions of 20µl, 10µl and 5µl were made in 1ml of sterile distilled water for each solution. All the solutions were prepared in the lab under aseptic conditions.

Lymphocyte Separation

Three ml of blood from a voluntary healthy young female donor was obtained after pre-information and consent. It was diluted with an equal amount of 1X PBS and layered over 3ml LSM (Hi Media) and centrifuged to separate the WBC layer. This was collected, washed with PBS twice and the cells were counted in a Neubauer's chamber as followed by khanna *et al.*[22] The cell suspension so obtained was appropriately diluted with cell culture medium 199(Hi Media), so that the final count was 2.45×10^4 cells/ml.

MTT Assay for proliferation

Basically, the method of *Mossmann et al* (20), *Barnabé* (21) and *khanna et al* (22) was followed. The yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by living cells by the action of mitochondrial enzymes such as succinate dehydrogenase to produce such compounds as NADH and NADPH resulting in the formation of purple formazan crystals inside the cells. The cells are then treated with DMSO or other organic solvents so that they release the purple compound. The OD is then read spectrophotometrically in an ELISA plate reader at 570-600nm. The amount of purple pigment formed is directly proportional to the number of living cells and therefore serves as a proliferation index.

Procedure of MTT Assay for this investigation

The proliferation/viability assay was conducted in 96 wells plates which were loaded with test solution, cells and media. Some wells contained cells and media which acted as controls while others had cells + media (180µl) + 20µl of appropriate concentrations of the insecticides / *Dashparni ark* / *Neem* oil emulsion. Some wells had only 20µl of these test solutions + 180µl of distilled water, for the determination of OD of only the test solutions. After loading the plate, it was kept for 4 hours in a humid incubator at 37°C. Then 20µl of MTT (5mg/ml in PBS) was added to each well and again the plate was incubated for 2 hours. Then 100µl of DMSO was added to each well and the plate was left for development of purple color in the incubator for 1 hour, after which the OD readings were taken in the ELISA plate reader at 600nm. The OD obtained for control wells was taken as 100% viability (for both 4 and 18 hours exposure) and the results of the treatments in terms of OD were expressed as % viability compared to the 100% viability of the control. Basically, the same procedure was repeated for 18 hours exposure. The results were subjected to statistical analysis using two-way ANOVA and students 't' test.

Trypan Blue Assay

Trypan blue assay is a rapid method to determine the number of living and dead cells present in a sample. When trypan blue dye is administrated in a sample of cells, the living cells, because they possess a complete and an undamaged cell membrane, do not take the colour of dye and remain white or colourless, however, the dead cells, because their cell membrane may be damaged, take up the colour of the dye and become blue, the procedure followed was that of Strober.(23) This test was performed for lymphocytes exposed to different concentrations of the insecticides for 45 min. in microtiter plate wells and their viability was compared to the relevant controls. Viability was calculated according to the formula given.

$$\text{Cell viability \%} = \frac{\text{Total number of living cells}}{\text{Sum of living and dead cells}} \times 100$$

Results

Table 1 shows that 4 hours incubation to Imidacloprid showed the maximum fall in viability at concentration of 1mM but the viability % shows an increase as the concentration of Imidacloprid increases, until it picks up to reach 75.15±3.43 at 12mM. Exposure to Dichlorovos for the same time period also shows the same trend of an initial steep fall in viability %, which picks up at 12mM to 95.55± 6.19. However, in the case of Profenofos, the initial fall very little, but the decrease in viability % increases with increasing concentration of the drug. The two-way ANOVA analysis of 4 hours exposure results show significant variations between treatments with different chemical insecticides ($p<0.01$). However, the variations within replicates were insignificant.

Table 1: The viability % of cells exposed to various concentrations of Imidacloprid, Profenofos and Dichlorvos for 4 hours

Average Viability % ± SD (For 4 hours)			
Concentrations	**Imidacloprid**	**Profenofos**	**Dichlorvos**
Control	100 ± 10.15	100 ± 10.15	100 ± 10.15
1mM	59.16 ± 5.36**	91.71 ± 6.54	76.98 ± 7.26*
4mM	63.46 ± 4.55**	88.59 ± 7.32	84.72 ± 7.72
8mM	65.11 ± 4.05**	82.93± 7.49*	86.73 ± 8.24
12mM	75.15 ± 3.43*	80.49 ± 6.19*	95.94 ± 6.19
* significance ($p<0.05$),** significance ($p<0.001$)			

For 18 hrs of exposure (Table 2) there was initial fall in viability at 1mM in all the three insecticides tested, (greater than that observed at 4 hrs) was followed by a rise in viability at higher concentrations. However, for Profenofos and Dichlorovos the highest values reached were greater than 100 % viability (control value). The two-way ANOVA analysis shows that the variation between treatments and between replicates is significant at 18 hours.

Table 2: The viability % of cells exposed to various concentrations of Imidacloprid, Profenofos and Dichlorvos for 18 hours

Average viability % ± SD (For 18 Hours)			
Concentrations	**Imidacloprid**	**Profenofos**	**Dichlorvos**
Control	100 ± 8.56	100 ± 8.56	100 ± 8.56
1mM	48.73± 7.10**	72.63 ± 6.70*	75.12 ± 7.14*
4mM	65.62± 9.74*	80.2 ± 9.75	79.18 ± 7.39
8mM	70.09 ± 8.77*	86.75 ± 7.11	85.5 ± 7.01*
12mM	76.09 ± 7.43*	103.32 ± 7.18	124.22 ± 7.65*
* significance ($p<0.01$), ** significance ($p<0.001$)			

Comparative values of viability at 4 & 18 hrs of exposure for the three insecticides

It is evident (**Figure 1A, 1B, 1C**) that for each insecticide the initial fall in viability at 1mM is greater at 18 hrs than at 4 hrs of exposure, but at higher concentrations the viability picks up. At 12mM, in Imidacloprid the viability is equal to the value at 4mM, but still less than the controls, in Profenofos it reaches above the control values. This trend is even more marked in Dichlorovos where the final viability % value at 12mM reaches 124.22(at 18 hrs).

Figure 1: Comparative values of viability at 4 & 18 hrs of exposure for the three insecticides

Figure 1(A) –Comparative viability of lymphocytes exposed to Imidacloprid for 4 and 18 hours

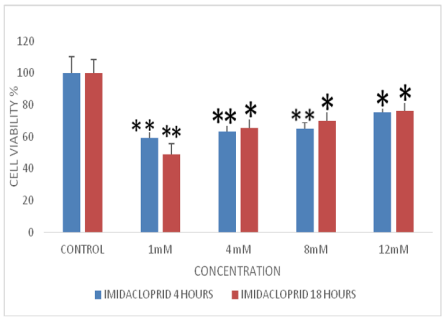


Figure 1(B) –Comparative viability of lymphocytes exposed to Profenofos for 4 and 18 hours

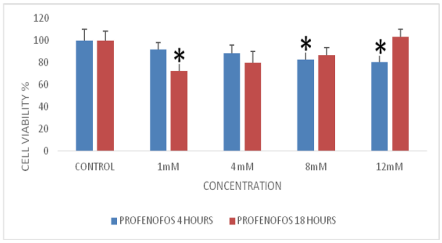
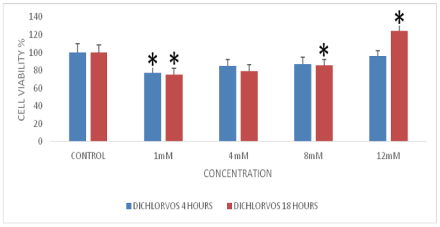


Figure 1(C) –Comparative viability of lymphocytes exposed to Dichlorvos for 4 and 18 hours



Dashparni Ark at 18 hours exposure

An increase in viability was evident at all concentrations. At 5 µl of the *dashparni ark*/ml of distilled water, it reached 127.68 ± 5.23 (significant at $p<0.01$). It again fell to 108.07 ± 5.40 at 20µl but the change was non-significant. So, in general *Dashparni Ark* is less toxic to cells and should be put to greater use. (Table 3).

At 4 hours exposure (Figure 2) in *Dashparni Ark*, the viability decreased at 5µl and 10µl / ml of distilled water but at 20µl /ml of distilled water, the viability increased above the control level. The changes at 5 µl (at 4 hrs) and 10µl (18 hrs) were statistically significant.

Table 3: The viability % of lymphocytes exposed to various concentrations of *Dashparni Ark* for 18 hours

Concentration (/ml dw)	Average viability % ± SD (For 18 Hours)
Control	100 ± 8.56
5µl	102.21 ± 4.66
10µl	127.68 ± 5.23*
20µl	108.07 ± 5.40
	* significance ($p<0.01$)

Figure 2: Comparative viability of lymphocytes exposed to *Dashparni ark* for 4 and 18 hours

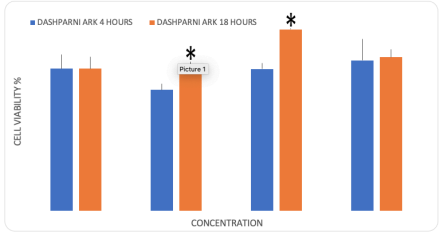


Figure 3: Comparative viability of lymphocytes exposed to *Neem* Oil Emulsion for 4 and 18 hours

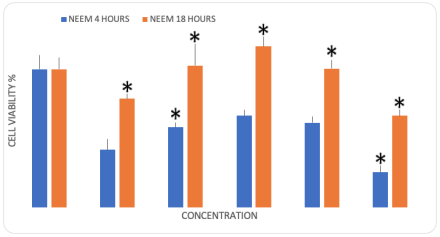
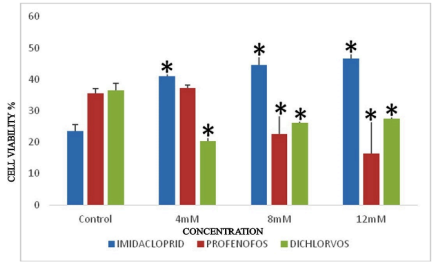


Figure 4: Comparative values of viability of cells exposed to various concentrations of Imidacloprid, Profenofos and Dichlorvos by Trypan blue assessment test.



Neem nano emulsion

In case of exposure of the cells to the various concentrations of *Neem* nano drop emulsion for 4 hours (Figure 3), a significant drop in viability was observed in all concentrations. This decrease was the greatest at 1.0mg/ml where the value recorded was 41.55 ± 7.7, but the viability gradually increased to 67.33 ± 4.41 at 1.5mg/ml. 1.7mg/ml was again toxic and the viability fell to 61.24 ± 4.32.

At 18 hours (Figure 3) exposure the values of viability showed an initial depression at 1.0mg/ml distilled water (significant at $p<0.01$) but at higher concentration the viability increased reaching a maximum of 116.70 ± 8.97 at 1.5mg/ml distilled water. However, the increase was not statistically significant. At 2.0mg/ml of distilled water, apparently the concentration became toxic to the cells and the viability decreased significantly to 66.40 ± 4.61.

The two-way ANOVA analysis of 4 hours exposure results show significant variations between treatments with different chemical insecticides, ($p<0.01$).

At 18 hours exposure it was found to have greater boosting effect for viability than that found in 4 hours exposures.

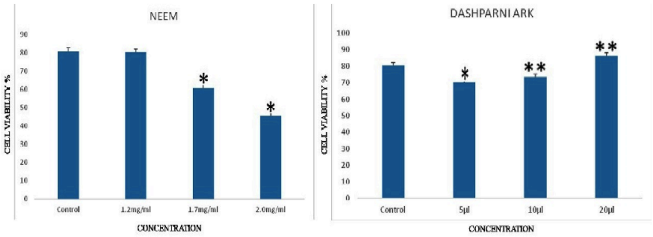
Trypan blue viability test

This was also applied to lymphocytes exposed to 4mM, 8mM and 12mM of the three inorganic insecticides for 45 minutes and the values were compared to control readings taken at beginning of experiment. All readings were taken in triplicate.

In the case of Imidacloprid, the viability increased significantly as compared to the controls and all the three concentrations. Profenofos treatment revealed an initial non-significant increase at 4mM exposure, but at higher concentrations a significant drop was observed. Dichlorvos treatment showed a significant drop in viability at all the three concentrations tested (Figure 4)

In the case of *Neem* nano emulsion, the trypan blue viability falls at three concentrations tested (1.2, 1.7 and 2.0 mg/ml). The decrease is significant at all concentrations except the first one (Figure 5).

Figure 5 - Viability of cells exposed to various concentrations of *Neem* oil nano emulsion and *Dashparni ark* by Trypan blue assessment test



In *Dashparni Ark*, the viability decreased at 5 and 10mg/ml distilled water but at 20mg/ml distilled water, the viability increased above the control level. All the changes were statistically significant (Figure 5).

Discussion

The treatments of lymphocytes by 1mM, 4mM, 8mM and 12mM concentrations of the insecticides for 4 hours shows a significant decrease in viability as compared to the control values. The decrease is more evident at lower concentrations but the viability values pick up at 8mM and 12mM. After 18 hours exposure, the fall is greater in every concentration probably because cells had more time to interact with their environment. Calderon – Segura *et al*(18) worked on trypan blue viability of several variants of Imidacloprid and found it to be toxic to lymphocytes in concentrations of 1.7 x 10^{-3} M to 2.0 x 10^{-3} M which are near to the concentrations we tested. Such results are supported by the work of *Kapoor et al* (24) who studied the effect of Imidacloprid on lipid peroxidation and antioxidant enzymes in rats. They concluded that Imidacloprid generates oxidative stress and induces changes at higher concentrations (5 and 10mg/day for 90 days) and not at lower ones. Perhaps the faster recovery of cells exposed to higher concentrations of drugs can be explained by the boosting effect on the expression of genes (such as CYP4C62, CYP4G15, GST1 and CYP303A1) regulating the cytochrome p450 detoxification pathway under these conditions. These genes were found to be expressed more frequently in Imidacloprid resistant insects.(25) Such findings are also supported by the work of *Tripathi et al* (26), who worked on the effect of Monocrotophos in the induction of cytochrome P450 enzymes in cultured rat brain cells. They found a time dependent increase in CYP2E1 – dependent NDMA – d, CYP2B – dependent PROD, when the cultured glial cells were exposed to 10^{-5}M of Monocrotophos. Time and dose dependent changes in striatal acetylcholine levels were studied in rats by *Karanth, S et al* (27).The animals were treated with chlorpyrifos (84,156 or 279 mg/kg) and were subjected to microdialysis conducted at 1,4 and 7 days after chlorpyrifos exposure for measurement of acetylcholine levels in striatum. Maximal increases in stratal acetylcholine levels were observed in rats receiving the maximum dose of the insecticide (279 mg/kg, 35 to 57 fold increase) increase was lesser at lower concentrations. Even higher cholinesterase levels were observed when an exogenous cholinesterase inhibitor was included in the perfusion buffer. Thus, the brain cells of the stratum respond with greater viability to produce higher amounts of cholinesterase (which is inhibited by the organophosphate) / acetylcholine at higher levels of exposure to chlorpyrifos. Thus, the work done by *Kapoor et al* (24), *Fajun Tian et al* (25), *Tripathi et al* (26) and *Karanth, S et al* (27) shows results which are parallel to our findings of increased viability of the cells at exposures to higher concentrations of the insecticides.

The IC_{50} of dichlorovos in HCT 116 (human colon carcinoma cells) was found to be 300 µM for 24 hours treatment.(28) The effect of Dichlorvos was studied on human lymphocytes by *Dean, B. J et al* (29) and he found that Dichlorvos had a cytotoxic effect on the cells at concentrations of 5 to 40µg/ml, but no significant cytogenetic changes were observed. It was observed by Perocco Paolo and Fini Angela (4) that it damages human lymphocytes DNA causing low DNA repair synthesis and it interferes with the process of reparative synthesis of DNA in lymphocytes exposed to UV rays. The cytotoxicity and genotoxicity of pesticide mixtures on lymphocytes was studied by *Asma Sultana Shaik et al* (30) and *Prabhavathy et al* (31) using chromosomal aberration test and comet test. The LC_{50} value for Profenofos alone was found to be 3.50µM (31) (which was near to the concentration used by us) and when used in combination with chlorpyrifos and endosulfan it was 1.8 and 2.0µM respectively. These values were lower than those used in this study but then their assay system was different. Thus, in general, in chemical insecticides the viability falls initially in these treatments but the values pick up as the concentration of the insecticide increases. These effects were more pronounced at 18 hours exposure.

Dashparni Ark for 18 hours seems to stimulate the proliferation at all concentrations. So, this extract of ten types of leaves fermented in cow dung and cow urine is a very eco-friendly, harmless insecticide which should be put to greater use by the farmers. For *Neem* Oil, this investigation has revealed that 1.5mg/ml exposure has a maximum beneficiary boosting effect on the viability of cell, but it was toxic at 2mg/ml.

However, *Neem* oil is not sprayed in form of nano drops so there is little cause for harm to the cells of sprayer. Work done on the effect of cypermethrin and *Neem* aqueous extract on lymphocytes by Khanna, A. *et al* (32) showed that aqueous *Neem* extract increases the viability of lymphocytes up to 196%. So, *Neem* extract was shown to promote viability of the cells. Thus, the application of organic insecticides seems to be a much safer alternative to the inorganic organophosphate insecticides tested in this investigation. The use of organic insecticides should be encouraged and the inorganic ones should be used with caution.

Conclusion

The *Neem* extract was shown to promote viability of the cells while *Dashparnik ark* produced very little damage, but at higher concentration it boosted the viability. Thus, the application of organic insecticides seems to be a much safer alternative to the inorganic organophosphate insecticides tested in this investigation. The use of organic insecticides should be encouraged and the inorganic ones should be used with caution, within safe limits and should be used when absolutely necessary.

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