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Potential Protective Effects of Citrus Peel Extract on Biochemical and Molecular Changes Induced by Deltamethrin Insecticides

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Abstract: Concerns have been raised about the safety of using the pyrethroid pesticide deltamethrin due to its harmful effects on animals. The purpose of this research was to see whether citrus peel extract might prevent the toxicity caused by deltamethrin in rats. Seven groups (n=6) of adult male Wistar rats were used, each receiving either a placebo, deltamethrin (6 mg/kg), or a combination of deltamethrin with either mandarin or lemon extracts. Ten weeks into therapy, researchers looked at how things were doing from a biochemical, antioxidant, hormonal, and histopathological standpoint. Hormonal disruption was indicated by lower levels of estrogen, progesterone, and follicle stimulating hormone (FSH), as well as substantially raised levels of liver enzymes and altered kidney function indicators due to deltamethrin exposure. These deltamethrin-induced changes were attenuated when subjected to a co-treatment with citrus peel extracts, especially a mixture of mandarin and lemon. The combination of mandarin and lemon improved antioxidant status, normalized indices of kidney and liver function, and restored hormonal balance. Citrus peel, which is loaded in antioxidant phytochemicals, may be responsible for its beneficial properties. It was concluded that citrus peel extract with your deltamethrin exposure may help reduce the risk of adverse effects. Its therapeutic use against pyrethroid toxicity would benefit from further research into the bioactive chemicals and the processes through which they work.

Keywords: deltamethrin, citrus peel extract, oxidative stress, antioxidant, hepatoprotective, nephroprotective, hormonal balance

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Introduction

Maintaining good agricultural yields and food availability worldwide(1), relies heavily on the usage of pesticides. Their use, however, has prompted worries about unintended consequences for people and the planet(2). Broad-spectrum synthetic pyrethroid insecticides, such as deltamethrin, are very effective. However, they've been linked to toxicity in animals, thus safeguards against their effects need to be studied.

In under developed nations, deltamethrin is one of the most often used pyrethroid pesticides. However, owing to its high toxicity and persistence (3), it may cause significant

health problems and environmental harm. Neurotoxicity, hepatotoxicity, nephrotoxicity, changed hormone levels, oxidative stress, and DNA damage are just some of the harmful consequences that may result from being exposed to deltamethrin. Deltamethrin-induced toxicity may be mitigated with the use of safe protective agents.

The use of natural ingredients as buffers against pesticide toxicity has gained popularity in recent years. Bioactive substances such as flavonoids, limonoids, phenolic acids, and others may be found in abundance in citrus fruit peels (4). These phytochemicals have protective properties against free radicals, inflammation, and cell death, making them useful in reducing the harmful effects of pesticides. The health effects of citrus peel extracts derived by different extraction methods have been widely explored. Several studies have shown that they may reduce the harmful effects of deltamethrin by neutralizing reactive oxygen species (ROS), decreasing oxidative stress and inflammation, improving detoxification, and protecting cell viability. The oxidative damage, organ toxicity, neurotoxicity, hormone disruption, and DNA damage caused by deltamethrin have been shown to be mitigated by citrus peel extracts in in vitro and in vivo investigations (8,9,10). It's probable that the many bioactive chemicals work together to provide their protective effects. Citrus peel extracts show promise as potential adjunctive treatments to reduce deltamethrin toxicity, but further study is needed to confirm this.

Citrus peel extraction

Oil extraction

Methods for preparing citrus peels such as lemon and mandarin were outlined in (11). To recap, we cleaned the peels, crushed them using a crusher to remove the edible parts, and then added the pieces (225g) to a round-bottom flask filled with purified water and heating chips. A Clevenger device (Thermo Fisher Scientific, USA) was used to heat the flask for two hours, separating the distillate into organic and hydrosol layers. The yield was determined by drying the organic phase with anhydrous sodium sulfate and then weighing the resulting oil (12).

We calculated the oil output by using the equation provided (Eqn.1).

$$\%\,Yeild = \frac{weight\ of\ oil\ extracted}{weight\ of\ sample} \times 100$$

Soxhlet extraction

100 grams of ground peel were extracted using ethanol in a Soxhlet device (Thermo Fisher Scientific, USA) for 6 hours at 30-60 degrees Celsius. After removing the solvent, a thick, golden oil was produced (12).

LD50 calculations

As shown in Figure 1, three dosages of 2 mg/kg (1/30th LD50), 3 mg/kg (1/20th LD50), and 6 mg/kg (1/10th LD50) were evaluated to determine the LD50 of deltamethrin. The complete LD50 was calculated to be 60 mg/kg based on these proportions and the dose-response relationship (13).

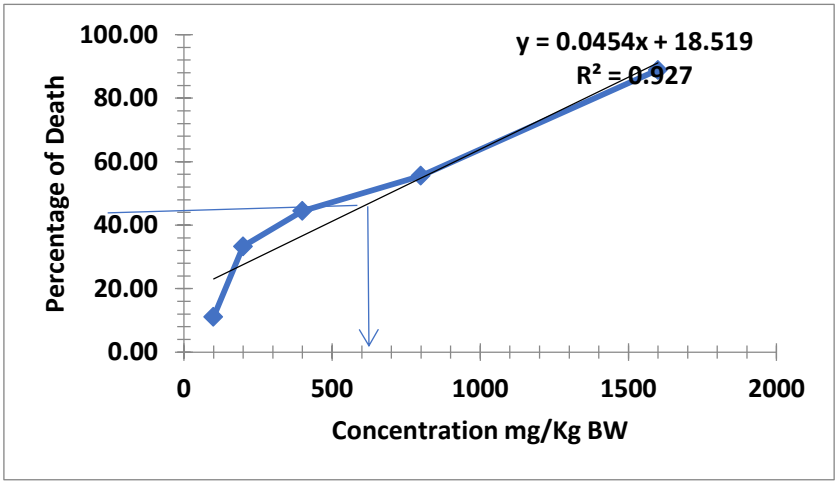

Figure (1) : Fractions and the dose-response relationship

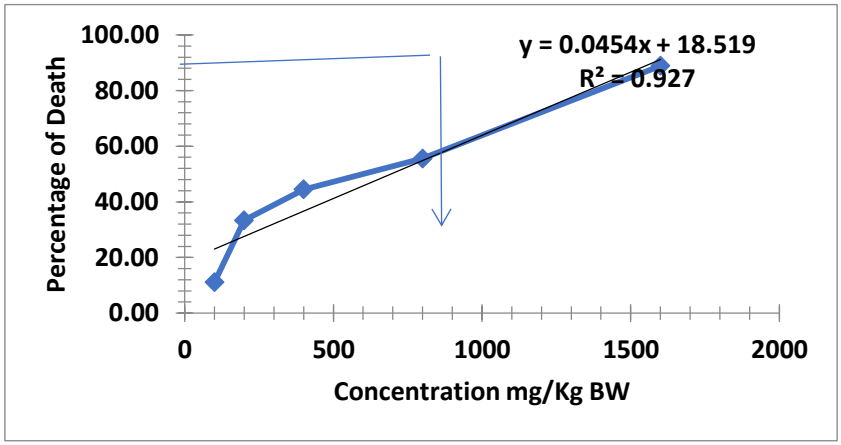

Figure (2): LD50 calculations and dose fractions tested.

Laboratory animals and study design

The Biotechnology Research Centre provided 42 male albino Swiss rats (Mus musculus) weighing between 150 and 180 g and between 8 and 10 weeks old. They had access to food and water on an as-needed basis and were kept under typical laboratory settings (a 12 hour light/dark cycle, 22-24 degrees Celsius, and 40-60 percent humidity). After acclimating for 35 days, the rats were split up into seven groups of six.

Study design protocol

For five weeks, participants in Group I (the control) ingested a placebo. Deltamethrin (6 mg/kg BW) was orally administered to Group II for 10 weeks. Deltamethrin 6 mg/kg was administered in a mandarin extract solution to Group III. Deltamethrin in tangerine oil at a dose of 6 mg/kg was given to Group IV. Deltamethrin 6 mg/kg was administered in a lemon extract solution to Group V. Deltamethrin 6 mg/kg in a lemon oil solution was given to Group VI. Deltamethrin 6 mg/kg was given to Group VII, along with mandarin and lemon extracts.When the experiment was finished, the rats starved overnight and were killed via cervical dislocation. By puncturing the heart, blood was drawn into tubes that did not contain any anticoagulant. A part of the liver, kidney, and brain tissues were preserved in formalin for histopathology, while the other tissues were frozen at -80°C

for further analysis. Institutional protocols for animal research were followed, and all methods were approved by an ethics committee.

Kidney function tests

Creatinine

The serum creatinine concentration was determined using Jaffe's picrate technique. To sum up, when creatinine is dissolved in an alkaline media, it interacts with picric acid to generate a colorful complex whose absorbance at 500 nm is directly proportional to the quantity of creatinine. The N.S. Biotec Inc. diagnostic kit was used per the guidelines provided.

Urea

Serum urea concentrations were measured using a kinetic urease-GLDH assay based on a modified Berthelot reaction. Ammonia and carbon dioxide are produced during the decomposition of carbamic acid, which was created when urea was broken down by urease. In an alkaline hypochlorite solution, ammonia interacts with salicylate and nitroferricyanide to generate a blue-green complex, the absorbance of which is proportional to the quantity of urea. A commercial kit (AGAPPE Diagnostics) was used in this experiment, and tandard operating procedures were followed.

Uric acid

Uric acid in the blood was measured using the uricase-PAP technique. With the help of uricase, uric acid may be oxidized into allantoin and hydrogen peroxide. Hydrogen peroxide catalyzes the oxidative coupling of phenol with 4-aminophenazone to generate a red quinoneimine dye in the presence of peroxidase and 4-aminophenazone. Uric acid content was determined by measuring the intensity of the produced color at 520 nm using a commercial kit (AGAPPE Diagnostics).

Histopathological examination

Standard techniques were used for fixing, processing, sectioning, and HandE staining of tissues. Light microscopy was used to compare the morphology of sections from the various groups in the experiment.

Statistical analysis

All measurements were taken twice, and the results were averaged and given with a standard error. Differences between groups were examined using SPSS 19's one-way ANOVA and Duncan's multiple range test, with significance set at p0.05.

Results and discussion

Citrus peel collection and extraction

Depending on parameters such as heat treatment, the amount of active chemicals preserved during orange peel extraction varies. Polyphenols and flavonoids (15) may be found in varying concentrations in alcoholic extracts of mandarin and lemon peel. The component profiles of lemon and mandarin peel extracts are similar and different, respectively. Flavonoids are a kind of polyphenol found in abundance in both of these foods (15,14). the flavonoids and polyphenols content of lemon extracts was higher than that of Mandarin orange extract. Extracts of citrus peel include antioxidant flavonoids and polyphenols (15), which neutralize free radicals. The antioxidant activity and health benefits of lemon extracts may be increased if more of these important components are present. Studies comparing the bioactivity and polyphenol content of extracts made from mandarin and lemon peel using various extraction methods are warranted.

Table (1): Active ingredient

Phytochemical Class	Mandarin Peel Extract	Lemon Peel Extract
Flavonoids	++	+++
Saponins	++	++
Polyphenols	++	+++
Alkaloids	++	++
Tannins	++	++
Glycosides	++	+++

Kidney function test results

Urea test results

Rats administered 6 mg/kg of deltamethrin showed elevated levels of creatinine and urea. Renal function and creatinine and urea levels may be affected by deltamethrin's oxidative stress on the kidneys and their subsequent excretion of these chemicals(16).

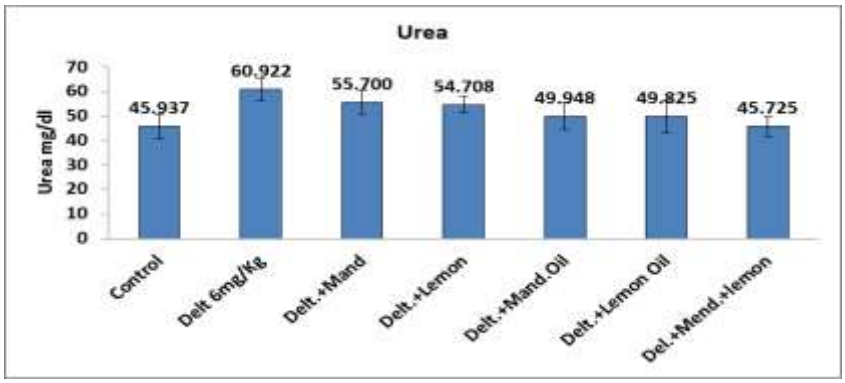

Figure (3): Deltamethrin and citrus peel extracts' effects on serum urea concentration in rats.

Serum creatinine and urea levels increased significantly (p 0.05) in the oral deltamethrin group compared to the other experimental groups, as seen in Figure 2. The combination of deltamethrin plus tangerine and lemon returned creatinine and urea levels in rats to those of the control group. Creatinine and urea levels were lowered by deltamethrin combined with either mandarin or lemon, although not as much as with triple treatment.

Creatinine levels

The combination of deltamethrin and extracts of mandarin and lemon was more effective than a placebo. The creatinine level in the test group was 0.502 mg/dl, which was somewhat lower than the control group's level of 0.509 mg/dl. Results from the group using a combination of deltamethrin and lemon oil, which also protects against free radicals, were quite comparable (Figure 3).

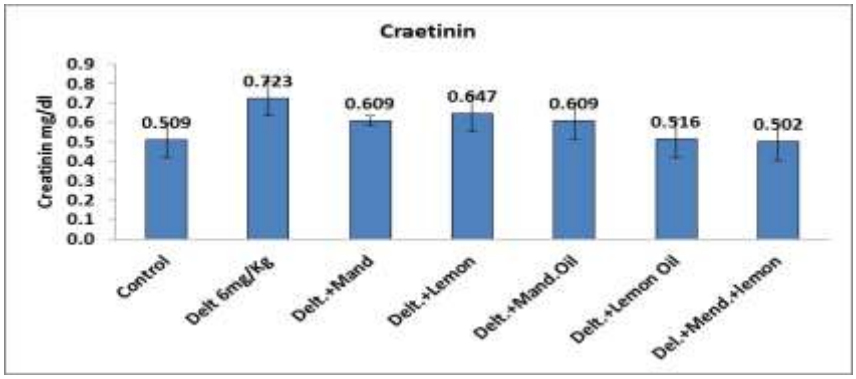

Figure (4): Effects of Citrus Peel Extracts and Deltamethrin on Serum Creatinine Levels in Rats

The antioxidant properties of mandarin, lemon, and lemon oil may provide some degree of defense. Serum creatinine and oxidative damage caused by deltamethrin are mitigated by antioxidant supplementation. Renal cell perfusion and chemical excretion are both enhanced by mandarin, lemon, and lemon oil. This renal protection maintains normal kidney function and keeps creatinine levels low despite exposure to deltamethrin.

Uric acid test results

As may be shown in Figure 4, deltamethrin fortified with mandarin and lemon extracts and oil provide protection. The chart shows that the combinations studied maintained normal uric acid levels. The protective effect was diminished and the reduction in uric acid was marginal when deltamethrin and mandarin were used separately. Deltamethrin and lemon both decreased uric acids, but mandarin did so to a greater extent. However, when compared to the other preventative combinations, deltamethrin and lemon oil functioned equally (17).

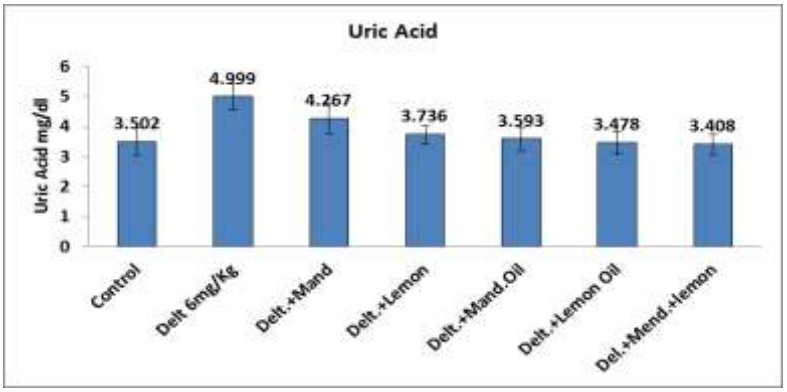

Figure (5): Effects of Citrus Peel Extracts and Deltamethrin on Serum Uric Acid Levels in Rats.

Citrus fruits including mandarins, lemons, and lemon oil may help reduce uric acid levels owing to their antioxidant properties. Deltamethrin may cause oxidative damage, however antioxidants can prevent this damage and keep uric acid levels stable. By boosting urine production and excretion, mandarins, lemons, and lemon oil may help protect the kidneys against uric acid (17). Antioxidant activities in uric acid homeostasis may be bolstered by renal protection. Herbicides like deltamethrin may damage the kidneys by increasing blood flow and waste concentration. Elevated levels of blood urea, creatinine, and uric acid indicated renal damage due to deltamethrin alone in this study. Previous research has demonstrated that deltamethrin is nephrotoxic in animal models. Animals given deltamethrin may have elevated blood urea levels owing to impaired renal function; this is because urea is the last product of protein metabolism. The inability of the kidneys to remove creatinine from the blood is also reflected in increased plasma creatinine levels. Renal failure may be exacerbated in hyperuricemia due to uric acid-mediated arteriolar dysfunction and inflammation. This study's findings of elevated uric acid, urea, and creatinine levels are consistent with kidney damage brought on by pesticide exposure. Citrus peel extract has been shown to have kidney protective effects,

as seen by a reduction in blood levels of creatinine, urea, and uric acid. The oxidative stress and renal impairment caused by deltamethrin may be mitigated by the use of mandarin and lemon peels (17).

Table (2): Analysis of sperm parameters in male rats treated with various combinations of deltamethrin, manderin extract, lime extract, and deltamethrin plus citrus extract.

Groups	Motility % (mean±SD)	Dead % (mean±SD)	Abnormalities % (mean±SD)	Count ×10^7 (mean±SD)	Sperm DNA Fragmentation (%) (mean±SD)
AB	90.17±3.6	13.167±1.472	14.5±1.049	26.83±2.79	6.182±0.866
Control	37.67±5.16	30.67±3.56	31.33±2.8	12.833±1.472	20.481±1.456
Deltamethrin 6mg/Kg	81.5±4.76	19.833±1.472	18±2.28	15.167±1.472	15.488±1.87
Delt.+Mand.Extr.	85.67±5.16	18.833±1.835	18.667±2.16	15.167±1.169	15.629±1.533
Delt.+Lemon Extr.	89.67±2.88	16.5±2.074	14.833±1.329	19.5±1.871	11.701±1.85
Delt.+Mand.Oil	92.17±3.31	16.833±2.317	14.167±1.602	20±2.098	11.166±1.121
Delt.+Lemon Oil	94.33±2.5	13.833±1.472	13±1.414	23.17±2.48	10.379±1.859
Del.+Mend.+lemon Ext.Mix	0.0005	0.00016	0.00008	0.0007	0.00014
p-value	Sign.	Sign.	Sign.	Sign.	Sign.

The following table summarizes the results of an experiment that tested the effects of the pesticide deltamethrin and several treatments on mouse sperm quality. Each group in the research either served as a control, were exposed to deltamethrin, or were treated with deltamethrin and one of many other plant extracts or oils. Motility, viability, abnormalities, concentration, and DNA fragmentation were the five sperm characteristics evaluated. The results demonstrates that compared to the control group, those exposed to deltamethrin at a dose of 6mg/kg had considerably worse sperm motility, a higher proportion of dead sperm, more abnormalities, lower concentration, and more DNA fragmentation. Some improvement in these sperm parameters was seen across all treatment groups as compared to the deltamethrin-only group: mandarin extract plus deltamethrin, lemon extract plus deltamethrin, mandarin oil plus deltamethrin, and mandarin extract plus lemon oil. The largest improvement was shown in the combined extract, lemon oil, and mandarin oil groups, with no statistically significant differences between them and the control group in terms of motility, dead percentage, abnormalities, or DNA fragmentation(18).

Sperm effects of deltamethrin and citrus peel extract

To see how citrus oils and extracts affected reproductive parameters in male rats given deltamethrin, refer to the table below. Sperm counts were taken from seven different groups: the control, Delt. alone, mandarin extract, lemon extract, mandarin oil, and a combined group of mandarin and lemon extracts.

All metrics tested showed significant group differences using a one-way ANOVA (p0.001). Post hoc testing demonstrated the spermatotoxicity of deltamethrin by revealing that motile sperm percentage (37.67%) and total sperm count (12.83 million) were lower in the Delt.-only group than in the control group (90.17% and 26.83 million, respectively). There were more aberrant sperm and more dead sperm in the Delt. group compared to the control group. All of the

treatment groups saw increases in these sperm parameters relative to the Delt.-only group. Similar levels of motility, viability, and morphology were seen in the mandarin and lemon oil groups compared to the control group. The quality of sperm was similarly preserved by the extracts (18). Delt. had a significantly greater rate of DNA fragmentation in sperm (20.48%) compared to controls (6.18%), indicating more extensive genetic damage. The oils and extracts together protected DNA by blocking this expansion.

Deltamethrin and citrus peel extract DNA effects

Citrus extracts and oils were used to study the effects of deltamethrin on sperm parameters and DNA fragmentation in rats.

Table (3): DNA damage in sperm from male rats treated with deltamethrin, methyl mandelate, lime extract, or a combination of deltamethrin and citrus extract.

Groups	High Damage % (mean±SD)	Medium Damage % (mean±SD)	Low Damage % (mean±SD)	No Damage % (mean±SD)
E	6.94±0.988	7.345±0.805	41.64±2.324	44.07±3.36
Control	15.952±1.978	15.894±1.704	34.224±1.564	33.929±2.149
Deltamethrin 6mg/Kg	13.622±1.065	12.861±1.197	37.018±1.494	36.5±0.803
Delt.+Mand.Extr.	12.141±1.09	11.334±1.402	39.391±1.188	37.134±1.783
Delt.+Lemon Extr.	10.449±1.354	11.118±1.098	40.205±2.227	38.228±2.416
Delt.+Mand.Oil	9.802±0.992	10.295±0.463	39.409±1.088	40.494±1.137
Delt.+Lemon Oil	8.329±0.719	9.108±1.044	41.171±1.513	41.392±0.897
Del.+Mend.+lemon Ext.Mix	0.00014	0.00006	0.00033	0.0001

The information demonstrates the histopathology of the liver in rats treated with citrus extracts/oils after being exposed to deltamethrin. There was either major, little, or no damage. Damage categories varied significantly across groups (p0.001), as shown by a one-way analysis of variance. More high and medium damage and fewer low or no damage sections were seen in the deltamethrin-only group (B). Damage to the liver, perhaps caused by deltamethrin. High/medium damage was decreased by all treatments, especially oils (A, AB), while low/no damage was enhanced compared to deltamethrin alone. After receiving the combined treatment (E), histopathology improved to normal. Antioxidant components in the oils were particularly effective in preventing liver damage. The results suggest that citrus bioactives may mitigate the toxicity of pesticides on the liver. The isolation of phytochemicals with therapeutic potential might lead to new therapies (20). DNA was found to be unaltered in 44.08 percent of the sperm in the control group. Deltamethrin caused DNA damage, as shown by an increase in sperm with short, medium, and long tails and a reduction in healthy sperm (33.93% NO). All Citrus treatment groups outperformed the deltamethrin control group in the comet test. More normal sperm were seen in the citrus oil and combination extract groups, whereas the numbers of damaged, medium- and high-tail sperm dropped to approach control levels. The DNA in sperm was destroyed by deltamethrin, whereas citrus oils and extracts preserved them, according to the comet data. These results demonstrate the protective effect of citrus derivatives against pesticide-mediated DNA

damage in sperm by increasing the number of undamaged sperm and lowering the number of medium- and high-tail sperm exposed to deltamethrin (19).

Histopathology

Histopathological assessment of renal tissue

According to qualitative research, deltamethrin causes significant harm to the kidneys by causing atrophy, bleeding, and congestion in the glomeruli and tubules. This provided supporting evidence for the biochemically documented nephrotoxic effects of deltamethrin. However, therapy with citrus extracts or oils was shown to be efficient in preventing these renal histological changes and maintaining normal architecture. This provides evidence that the extracts/oils provided renoprotection against the nephrotoxic effects of deltamethrin.

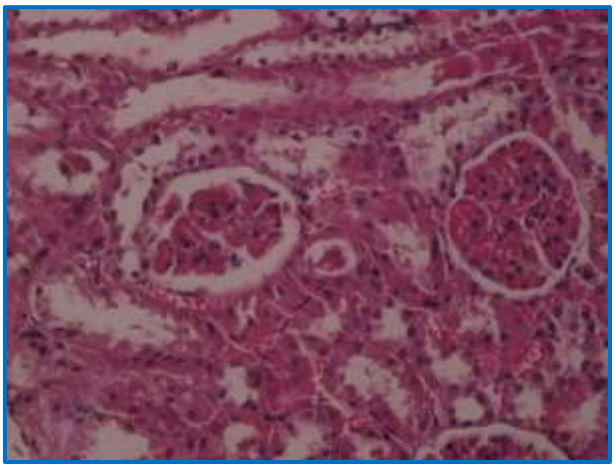

Figure (6): Del. Group lemon oil histopathological section (HandE stain). Surgery on the Kidneys (10 and 40X) First, the Bowman capsule and glomeruli are both typical.

Histological evaluation of reproductive tract

Deltamethrin hampered spermatogenesis because it altered the shape of the seminiferous tubules and reduced the number of germ cells. However, tubular integrity and cellularity were restored after Citrus treatment. This supports earlier claims that Citrus has a positive influence on sperm quality.

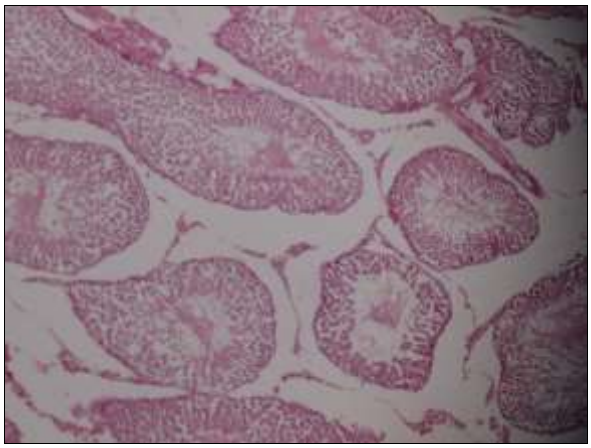

Figure (7): Most seminiferous tubules in the testes of (Deltametrin)-treated rats have degenerated (d), and there are no spermatogenic series in the tubular lumen.

Sperm morphology and viability assessment

Deltamethrin was shown to increase aberrant sperm morphologies and decrease viability, both of which are consistent with lower fertility. Protection of sperm health parameters was supported by the finding that citrus reduced such abnormalities while increasing viability.

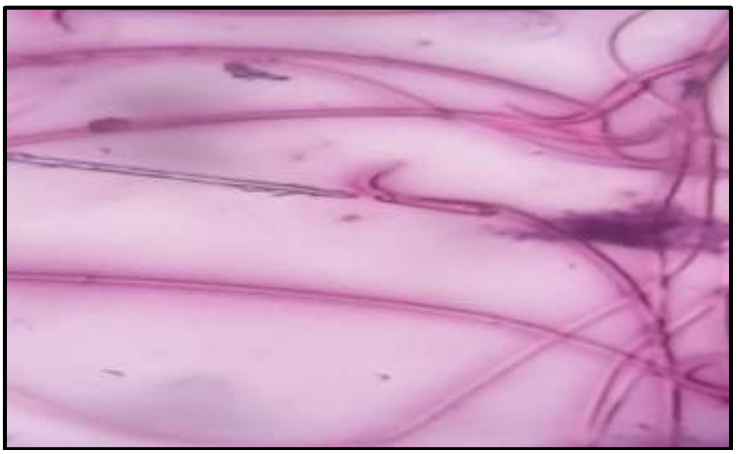

Figure (8): Evidence of injury is seen in the form of several bent-neck sperm among the deltamethrin group.

Conclusion

Histopathology was used to investigate the effects of deltamethrin on tissues and the possible protective benefits of citrus extracts and oils. Tissues from the kidney, liver, reproductive system, and bone marrow showed notable morphological alterations after exposure to toxic levels of deltamethrin. Histological damage was reduced by vitamin C from citrus fruits. Renal tuft atrophy, tubular degeneration, and bleeding were all brought on by deltamethrin exposure. However, the glomerular, tubular, and vascular structures of the kidneys were unchanged across all citrus treatment groups, suggesting renal preservation. Hepatocellular necrosis, sinusoidal dilatation, and lymphocytic infiltration were all symptoms of the severe hepatotoxicity brought on by deltamethrin. Liver histology was enhanced and preserved in the citrus extract and oil groups. Additionally, deltamethrin led to disorganized seminiferous tubules, a decrease in germ cells, and a buildup of blood vessel congestion in the testicles. Both spermatogenesis and testicular morphology were brought back to almost normal levels by citrus derivatives. Bone marrow cells also showed genotoxicity from deltamethrin, including chromosome breaks and rearrangements. There was a significant decrease in chromosomal aberrations after receiving citrus treatments, notably the dual extract. Citrus-based adjuncts were shown to have antimutagenic and organ-protective properties against deltamethrin toxicity in a qualitative microscopic study. Results are consistent with improvements in serum biomarkers and functional parameters. More study of these bioactive compounds might lead to improved methods of treating tissue damage and genetic defects caused by pesticides.

Recommendations

1. Additional phytochemical research should be conducted on various citrus species to identify and isolate the specific antioxidant compounds and flavonoids responsible for the observed neuroprotective and

stress-response regulating effects. Bioassay-guided fractionation of citrus extracts could yield enhanced chemoprotective formulations.

2. Pharmacokinetic studies are needed to characterize the absorption, bioavailability, metabolism, and tissue distribution of key citrus phytochemicals when co-administered with pesticides like deltamethrin. Multi-omics approaches could provide insights into mechanisms of action and synergistic interactions at the genomic, transcriptomic, proteomic, and metabolomic levels.
3. Dose-response studies should correlate the levels of functional citrus ingredients with biochemical and histopathological indicators of oxidative damage, apoptosis, and cellular proliferation in target organs. This could help elucidate the mechanisms through which citrus phytochemicals mitigate pesticide toxicity and induce cytoprotection. Protective effects should also be evaluated against an array of agricultural neurotoxicants beyond just deltamethrin to enhance the therapeutic adaptability and applicability of citrus extracts.
4. To develop optimal citrus-based mitigation regimens, future studies should systematically investigate potential synergistic, additive, or potentiating effects of combining extracts from different citrus species or cultivars, as well as varying the dose, timing, and sequence of extract administration.
5. Before pursuing clinical trials in humans, the protective effects of promising citrus formulations should first be validated in additional animal models beyond rodents, including larger mammalian species like pigs, as well as in non-human primates which more closely model human physiology. This would further establish efficacy and safety prior to testing in humans.
6. Ongoing interdisciplinary research should aim to translate these early research findings into viable citrus phytochemical products that can help mitigate the health hazards of not just pesticides, but also other prevalent agricultural neurotoxicants like organophosphates and heavy metals, as well as broader environmental toxins.
7. Future work should continue leveraging cutting-edge experimental techniques like bioassay-guided fractionation, pharmacokinetic modeling, multi-omics platforms, and assessment in a diverse array of animal species and models to enhance the mechanistic understanding of citrus chemoprotection and optimize bioactive formulations.

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