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ORIGINAL ARTICLE

The hepatoprotective potential of nigella sativa oil against cypermethrin-induced hepatotoxicity in rats: A histomorphological investigation.

Nayyab Khattak[1], Noman Ullah Wazir[2], Mohammad Saeed[3], Ayesha Iftikhar[4], Rabbia Jabbar[5], Muhammad Saleh Faisal[6]

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ABSTRACT… Objective: To determine the hepatotoxic effects induced by cypermethrin. The study also attempts to evaluate the hepatoprotective efficacy of Nigella sativa oil against hepatotoxicity produced by cypermethrin. **Study Design:** Experimental study. **Setting:** Department of Anatomy, Pharmacology, and Pathology of Peshawar Medical College and Animal house of PCSIR Laboratories Complex, Peshawar. **Period:** May 2023 to Dec 2023. **Methods:** Animals were divided into two groups: Control (Group A) and Experimental (Group B). The Experimental group (Group B) is divided into two subgroups Group B-I and Group B-II. Forty-two male Sprague Dawley rats were randomly assigned to three groups of fourteen rats each in order to conduct an experimental investigation. Group A served as a control group. Cypermethrin (5.5mg/kg^{-1} body weight) per day dissolved in corn oil was administered orally to Group B-I. Cypermethrin (5.5 mg/kg^{-1} body weight) per day orally and (1ml/kg^{-1} body weight) per day of Nigella sativa oil were given to group B-II. Liver obtained from these groups were fixed for histological studies under light microscopy. Liver tissues of the cypermethrin-treated animals showed leucocytic infiltration, hydropic alterations such as ballooning degeneration and focal lytic (spotty) liver necrosis. **Results:** After four weeks of treatment with cypermethrin demonstrated significant liver damage in group B-I animals. In group B-II Nigella sativa oil significantly reduced hepatotoxicity and induced regenerative changes. Nigella sativa oil demonstrated protection against the cypermethrin and preserved the normal histological architecture of the liver. **Conclusion:** Cypermethrin had a hepatotoxic potential and caused hepatotoxicity in liver, as evidenced by the histological changes in the liver tissue. However, the study also found that Nigella sativa oil showed a better hepatoprotective effect, as it was able to effectively reverse the liver damage caused by both cypermethrin.

Key words: Cypermethrin, Hepatocytes Ballooning Degeneration, Hepatotoxicity, Liver Focal Necrosis, Nigella Sativa.

INTRODUCTION

Pesticide usage has become frequent and popular nowadays, and its harmful effects have given rise to severe health problems. When insecticides are utilized on crops, they can leave behind residues. Over time, these residues have the potential to accumulate in the body, possibly resulting in health issues.[1] Currently, 30% of insecticides used worldwide are synthetic pyrethroids, which are highly effective insecticides.[1] They originate from pyrethrins, naturally occurring insecticidal substances present in chrysanthemum flowers. Pyrethroids find widespread application in agriculture, household insecticides, pet care products, and mosquito management.[2] They are preferred over older insecticides due to their relatively lower toxicity to mammals and birds. Nonetheless, improper usage can still pose risks to aquatic organisms and beneficial insects.[3]

Pyrethroid pesticides can be generically classified as type I or type II based on whether an alpha-cyano group is present or absent, respectively.[3] Cypermethrin is categorized as a type II pyrethroid insecticide, which is widely utilized in developed and developing nations for many purposes related to pest management.[4] It can enter the body through ingestion, inhalation, and absorption through the skin.[5,6] Chronic exposure to cypermethrin, due to its lipophilic

1. MBBS, M.Phil, Lecturer Anatomy, Peshawar Medical College, Peshawar.
2. MBBS, M.Phil, Ph.D, Associate Professor Anatomy, Peshawar Medical College, Peshawar.
3. MBBS, M.Phil, Professor Anatomy, Peshawar Medical College, Peshawar.
4. MBBS, M.Phil, Assistant Professor Pharmacology, Rehman Medical College, Peshawar.
5. BDS, M.Phil, Senior Lecturer Pharmacology, Rehman College of Dentistry, Peshawar.
6. MBBS, M.Phil, Ph.D, CHPE, CHR, Assistant Professor Pharmacology, Khyber Medical College, Peshawar.

Correspondence Address:
Dr. Noman Ullah Wazir.
Department of Anatomy
Peshawar Medical College, Peshawar.
dr.noman.wazir@gmail.com

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nature, causes it to store in lipid-rich body tissues and organs such as skin, body fat, liver, kidneys, ovaries and the neurological system of the central and peripheral regions.[6] When cyclomethrin penetrates the blood-brain barrier, it causes neurotoxicity and motor impairments. Ataxia, hyperreactivity, tremors, paresthesia, fatigue, hypersalivation, vomiting, diarrhoea, and urine incontinence are the most commonly reported signs of cypermethrin poisoning that impact the neurological and muscle systems.[6] Cypermethrin has been shown in several investigations to have a harmful impact on the liver and kidneys, among other mammalian organs. It has been found that cypermethrin affects the amount of marker parameters associated with the kidneys and liver in experimental animals. Since liver is the primary organ where pesticide metabolism occurs, cypermethrin is a hepatotoxic pesticide.[7-8] These chemicals cause the production of free radicals, which worsen oxidative stress in animals.[7-8] The body has defense mechanisms in place to prevent the harmful effects of oxidative stress, also known as reactive oxygen species (ROS), which include several different types of antioxidants.[9] Significant antioxidant substances comprise several vitamins, such as A, C, and E, many enzymes, such as catalases, glutathione reductase, superoxide dismutase, peroxidase, and minerals, such as zinc, copper, manganese, and selenium.[9] Other substances with antioxidant potential include uric acid, bilirubin, glutathione, and flavonoids.[9] The equilibrium between the produced free radicals and the antioxidant preventive defense system determines how much tissue damage reactive oxygen species (ROS) may bring about.[10] In the scientific community, nutraceuticals have lately gained a lot of popularity due to the strong correlation between a good diet and life expectancy.[10] Many nations in the eastern Mediterranean, northern Africa, the Indian subcontinent, and Southwest Asia produce Nigella sativa L. (NS, black cumin), a plant in the Ranunculaceae family.[11] The quinone components of N. sativa, which are present in both the fixed and essential oils, are largely responsible for the oil's efficacy. Thymoquinone (TQ), a significant bioactive component that makes up between 30 and 48% of the total components, plays a key role in the product's effectiveness.[12,11] Nigella sativa oil has been shown to have a wide range of beneficial and protective actions, including hepatoprotective, immune-modulating, anti-inflammatory, antioxidant, and analgesic. Back pain, asthma, fever, bronchitis, cough, chest congestion, dizziness, paralysis, chronic headache, inflammation, infertility, and various gastrointestinal disorders like dyspepsia, flatulence, diarrhea, and dysentery have all been treated with the plant's seed in traditional medicine.[11] Al-Bukhari quotes Prophet Muhammad (PUBH) as saying, "Use the black seed, which is a healing for all diseases except "As-Sam" which is DEATH.[13] Nevertheless, there are very few studies that include thorough histopathological analyses, looking at how Nigella sativa oil protects the liver against the harmful effects of cypermethrin.[11]

METHODS

The study was conducted in the department of Anatomy, Pharmacology, and Pathology of Peshawar Medical College and Animal house of PCSIR Laboratories Complex, Peshawar after ethical approval vide letter no: Prime/IRB/2023-383. The sample size for the experiment, using the G-Power Software with an effect size of 0.5, alpha set at 0.05, power set at 80%, with three groups, was calculated. The sample size required for the study was determined to be forty-two (fourteen rats in each group). In group A, rats were given standard food and water provided with water ad libitum. In group B-I, 5.5mg/kg of cypermethrin dissolved in corn oil[14] was administered daily via orogastric intubation for 4 weeks.[15] In group B-II, 5.5mg/kg of cypermethrin, dissolved in corn oil[14], and N. sativa oil (1ml/kg)[16] were administered daily via orogastric intubation for 4 weeks. Both the treatment groups were also fed on standard diet and provided with water as libitum. All the rats were weighed at the beginning of the experiment to calculate the amount of drug dosage required for administration. They were weighed again after one week to observe any changes in weight, and the drug dosages were then adjusted accordingly.

Animals were sacrificed for histological investigation after four weeks, and the liver was

taken right away and preserved for twenty-four hours in 10% neutral buffered formalin. The tissue was processed, embedded, sectioned, and stained by Hematoxylin & Eosin as well as Masson's Trichrome, in the histopathology laboratory of Peshawar Medical College, Peshawar to prepare them for examination under a light microscope (Olympus BX53) to search for the changes in the liver architecture. Specimens were fixed, dehydrated in an increasing sequence of ethyl alcohol, cleaned through two changes of xylene, infiltrated into three changes of molten paraffin wax (melting point: 58–60°C), and embedded in blocks of molten paraffin. The sections were mounted on sanitized slides after being cut into 5 micron-thick sections using a rotary microtome. Sections were stained with Hematoxylin and Eosin and Masson's trichrome for histopathological analysis.

Data was analyzed through social sciences (SPSS) version 22. Statistical result was given as mean and standard deviation for continuous variable. For the histology variables, frequencies and percentages were computed, and the chi-square test was used to determine the statistical significance of these categorical variables. A one-way ANOVA was used to examine the relationship between the continuous variables, and the Tuckey HSD test was used for multiple comparisons. Statistical significance was defined as a p-value of 0.05 or less.

RESULTS

In control group, no histopathological changes like ballooning degeneration, focal necrosis and confluent necrosis were seen. The liver histology was normal. Rats receiving cypermethrin i.e., in group B-I showed histopathological changes like ballooning degeneration, focal necrosis and confluent necrosis. Necro-inflammatory scores of histopathological parameters were given according to Modified histological activity index grading as shown in Figure-1.

All of the rats in group B-I showed the signs of moderate ballooning degeneration as shown in Figure-2-B. In the group B-II receiving the cypermethrin along with N. sativa oil revealed that 21.4% of the rats were having no signs of ballooning degeneration, while 28.6% of the rats showed focal ballooning degeneration, 35.7% showed mild, and 14.3% showed moderate ballooning degeneration as shown in Figure-2-C. In group B-I it was seen that 35.7% of the rats showed focal necrosis in one focus, 42.9% in 1 to 4 foci and 21.4% in 5 to 10 foci. In group B-II, it was observed that 71.4% of the rats having no focal necrosis while 21.4% showed in one focus and 7.1% in 1 to 4 foci, shown in Figure-2-E.

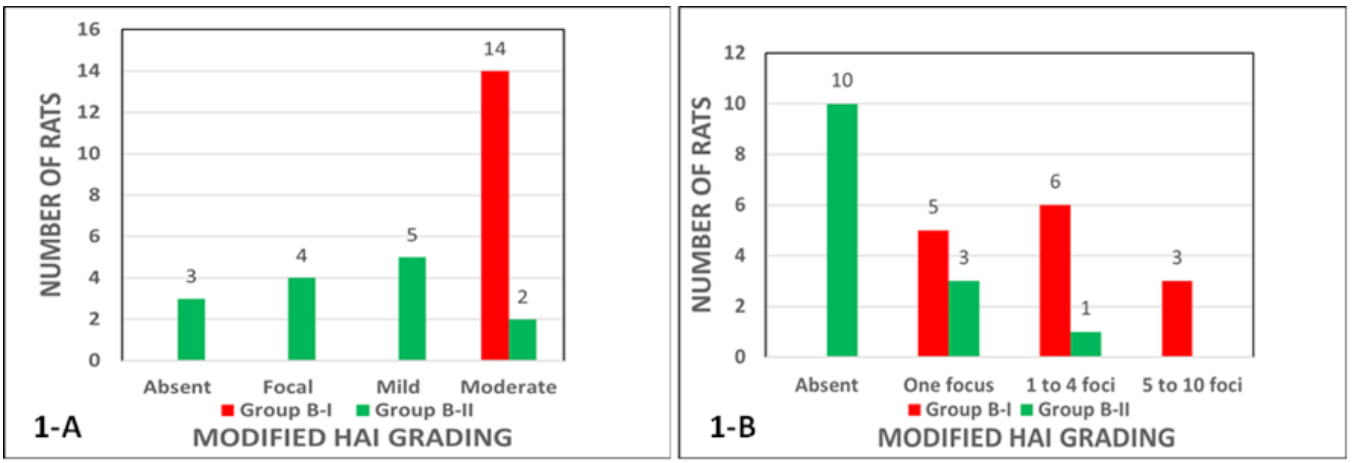

Figure-1. (1-A) Comparison of Group B-I and B-II showing ballooning degeneration. (1-B) Comparison of Group B-I and B-II showing focal necrosis.

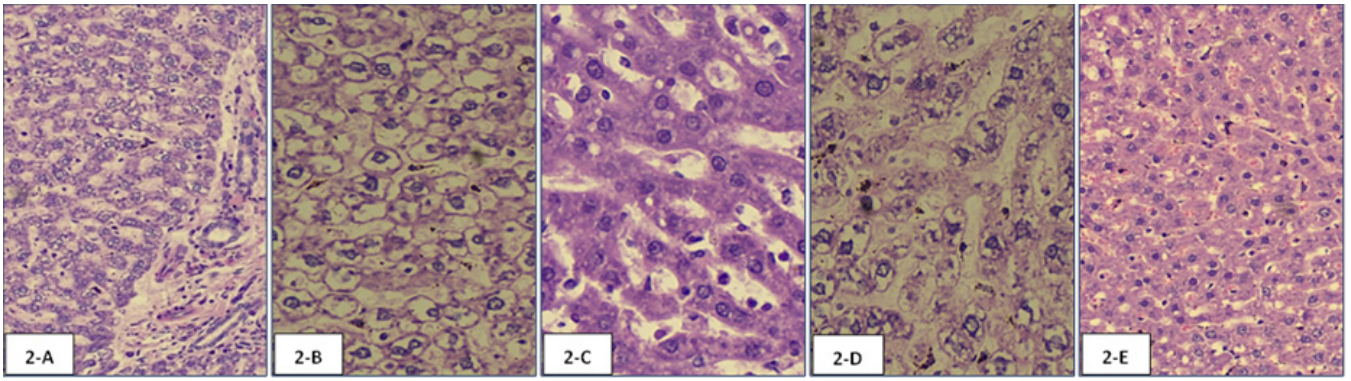

Figure-2. 5μm thick H&E-stained liver sections at 40X. (2-A) normal liver showing normal hepatocytes forming hepatic cords. (2-B) group B-I liver showing moderate ballooning degeneration in hepatocytes. (2-C) group B-II showing mild ballooning degeneration in hepatocytes. (2-D) group B-I liver showing moderate focal degeneration. (2-E) group B-II liver showing mild focal degeneration.

DISCUSSION

Unlike other pollutants, pesticides are very harmful substances that are purposefully added to the environment in large amounts.[17] Pesticides must typically be applied on a regular basis in traditional forms, such as concentrated emulsions, granules, or powders, and they must be sprayed or dusted directly into the necessary area.[17] These kinds of applications impact both terrestrial and aquatic ecosystems by causing pollution of the air, water, and soil, among other environmental issues.[18] In addition to indirect contact by ingestion of tainted water, vegetables, or meat, there can be direct contact with the pesticide through involuntary exposure or its application in treated zones (buildings, etc.).[18] Approximately 70% of Pakistan's population lives in villages, and the majority of them work in agriculture either directly or indirectly. Farmers use synthetic insecticides to keep pests away from their crops. The rate at which farmers are using synthetic pesticides is startlingly rising. Numerous other malpractices exist, such as spray painters who neglect to take the required safety measures. It results in dangerous mishaps and a host of issues, such as pesticide resistance, pesticide contamination, and the buildup of pesticide residues in both human and animal bodies. Furthermore, several studies have demonstrated that the majority of pesticides often used in agriculture have the potential to harm DNA. The antioxidative properties of Nigella sativa oil in hepatotoxicity signifies its capacity to alleviate liver injury induced by diverse toxic substances owing to its antioxidant attributes. Nigella sativa, recognized as black seed or black cumin, has a longstanding history in traditional medicine due to its manifold health advantages. Numerous studies have delved into the possible hepatoprotective effects of Nigella sativa oil, with particular emphasis on its antioxidative properties. This oil harbors bioactive compounds like thymoquinone, thymohydroquinone, and dithymoquinone, all known for their potent antioxidant capabilities.[7-8]

In the current study, it was discovered that cypermethrin exposure caused various histological changes in the livers of the rats. In the treated animals, ballooning degeneration and localized necrosis were observed in the liver parenchyma. These findings concur with those of Sirivastava et al., who observed that cypermethrin overdose in rats led to hepatic cell necrosis with pyknotic nuclei and hepatic laminae disarray in the hepatic structure. In male albino rats, repeated oral cypermethrin treatment resulted in numerous localized hepatic necrosis and hydropic degeneration of the hepatocytes.[19]

A similar finding of the focal necrosis has been reported in the study that demonstrates protective role of garlic extract and vitamin C against sub chronic toxicity of cypermethrin on the liver of rats.[20] Poonam et al., documented in their study the existence of focal areas of hepatocytes necrosis, showcasing the protective impact of Curculigo orchioides against the hematobiochemical abnormalities induced by subacute cypermethrin

toxicity in Wistarr rats.[21]

According to Hamid et al., the liver of Rattus norvegicus domestica rats given cypermethrin showed widespread hepatocyte vacuolar degeneration as well as a large number of hepatocytes with pyknotic and karyolytic nuclei.[22]

The current findings demonstrated that nigella sativa oil offers protection against cypermethrin's hepatotoxicity. The hepatic architecture of rats treated with nigella sativa oil and cypermethrin improved. According to Ebuehi et al., nigella sativa oil protected rats' livers from CCL4-induced damage by acting as an antioxidant and hepatoprotector.[13] Comparably, our result, which found reduced ballooning degeneration, is consistent with another study that highlights the hepatoprotective function of Nigella sativa oil against Bisophenol A.[23]

The combined administration of N. sativa oil and cypermethrin in our study significantly reduced the incidence of focal necrosis. This finding is in parallel with study done by Habeeb, regarding the hepatoprotective role of Nigella sativa oil versus iron stress in liver.[12]

According to Hosseini, treating rats with nigella sativa oil therapy has an antioxidant effect against the negative consequences of ethanol poisoning. They also mentioned that nigella sativa oil therapy resulted in a reduction of certain inflammatory cytokines and lipid peroxidation.[24] Mohamed also mentioned that the use of nigella sativa oil prevented the harmful effects of fipronil and improved the architecture of the liver. Therefore, the presence of nigella sativa oil's antioxidant qualities may be the cause of the studies observed hepatoprotective effects.[25]

CONCLUSION

The result of our study shows that cypermethrin at 5.5 mg/kg (body weight) produce significant changes in liver morphology due to production of free radicals and oxidative stress. These histomorphological changes include changes like ballooning degeneration in hepatocytes and focal necrosis. These hepatotoxic effects were found to be reduced by the concurrent use of antioxidant agent Nigella sativa oil. Hepatotoxicity, characterized by liver damage from toxins like alcohol, drugs, or environmental pollutants, is heavily influenced by oxidative stress. This stress arises from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses, potentially harming liver cells and tissues. Nigella sativa oil has demonstrated the ability to scavenge free radicals and diminish oxidative stress in the liver. Moreover, Nigella sativa oil's anti-inflammatory properties further bolster its hepatoprotective effects. As inflammation often accompanies hepatotoxicity and worsens liver damage, the oil's capacity to reduce inflammation aids in mitigating liver injury and facilitating recovery.

In conclusion, research suggests that Nigella sativa oil may provide substantial antioxidant and hepatoprotective benefits in hepatotoxicity cases. Nonetheless, additional clinical investigations are necessary to fully grasp its mechanisms of action and therapeutic potential in liver diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Nayyab Khattak	Concept, Data collection.	
2	Noman Ullah Wazir	Study design, Data analysis, Manuscript write-up.	
3	Mohammad Saeed	Statistical analysis, Bibliography.	
4	Ayesha Iftikhar	Data analysis and Curation.	
5	Rabbia Jabbar	Critical review, Editing final draft.	
6	Muhammad Saleh Faisal	Manuscript write-up, Data interpretation.