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PHYTOCHEMICAL PROFILING, *IN VITRO* AND *IN VIVO* SINGLE AND SYNERGISTIC CYTOTOXIC, ANTI-HEMOLYTIC, ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF *NIGELLA SATIVA*, *OLEAEUROPEA*, AND *ALLIUM SATIVUM*

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ABSTRACT

Background: The liver is essential for preserving metabolic homeostasis and protecting cells from oxidative stress-related harm. Natural treatments made from medicinal plants have attracted a lot of attention recently because of their possible hepatoprotective, anti-hemolytic, cytotoxic and antioxidant qualities. This study investigated at the individual as well as synergetic impacts of *Nigella sativa*, *Oleaeuropea*, and *Allium sativum* to unravel the intricate biological effects in both *in vivo*&*in vitro* settings.

Method: The research involved comprehensive phytochemical profiling of plants to identify bioactive chemicals, evaluating cytotoxic effects using in vitro techniques, assessing anti-hemolytic properties, and examining antioxidant properties of the extracts. To shed light on the oxidative stress and hepatoprotective properties of these plants randomized controlled trial was carried out on 42 albino mice. The mice were randomly divided into7 groups with 6 each. Hepatoxicity was induced by CCL4. The first group was given CMC; second group was treated withsilymarin (200mg/kg). The 3rd, 4th, and 5th groups were treated with plant extract nigella sativa, Allium sativum and Oleaeuropea respectively (200mg/kg). The last group was treated with combined extract of these plants (100mg/kg).

Results: The findings of this study demonstrated that there was significant anti-oxidant and hepatoprotective activity. Significant reduction was noted in ALT, AST and ALP. Furthermore, these plants extract also produced serum protein such as albumin & glutathione peroxidase level.

Conclusion: The findings of this study made a substantial contribution to the complex interactions between the biological activities displayed by these plants and their phytochemical composition.

Keywords: Nigella sativa, Oleaeuropea, Allium sativum, Hepatoprotective, Antioxidant, Anti-hemolytic

INTRODUCTION

For millennia, people all over the world have harnessed *N. sativa* seeds and oil to treat a diverse array of illnesses. Additionally, It holds a significant position in Indian traditional medical systems like Ayurveda & Unani(Wahab&Alsayari, 2023; Ahmad et al., 2013). *N. sativa's* therapeutic properties have been proven in ayurveda, Chinese medicine, Unani, and other medical systems (Ahmad et al.,

2013). The use of *Nigella* dates back over two millennia. Its inclusion in Greek medical literature confirms that it was a significant component of traditional medical approaches, even those employed by the Egyptians. It is clear from the discovery of the black seed in Pharaoh Tutankhamun's tomb that it was significant to Egyptian customs in the past (Padhye et al., 2008). The Old Testament book of Isaiah contains the first historical allusions to the Black Seed (Ibn Sina, 2007).

Oleaeuropea belonging to family oleaceae is a small tree and native to warm temperature region of world. In a situation of spiritual importance, the olive tree fruit are narrated over many historical periods in the Holy Quran& the Bible(Parvaiz et al., 2013). This tree has extensive history of nutritional values and therapeutic history. Olive oil is used for physical fitness according to ancient Greek literature. Olive oil has been utilized for many millennia in meal preparation, illumination, sacrifice offerings, ointment, & anointing for priestly or regal professions (Hashmi et al., 2015). In Islamic medicine, its importance has long been recognized. Allah, the Almighty, declares in the Holy Quran: "And a tree (olive) that springs forth from Mount Sinai, that grows oil, and (it is a) relish for the eaters (Ali, 2011)."

Throughout history, *Allium sativum* has been served with a variety of biological activities. It belongs to liliacae family. The medicinal qualities of garlic are attributed to its increased concentration of sulphur compounds, specifically diallyl disulphide, diallyl trisulfide, S-allyl cysteine, and allicin. It has long been recognised as a healthy spice and a well-liked remedy for a range of illnesses and physiological issues (Tesfaye&Mengesha2015; Singh R &Singh K 2019).

This study aims to close this knowledge gap by performing a comprehensive phytochemical profile of *Allium sativum*, *Oleaeuropea*, and *Nigella sativa*. The investigation goes beyond simple identification to better understand the combined and individual-agent cytotoxic effects that these plants display, both *in vivo* and *in vitro*. A crucial component of my research involves comprehending the apoptotic pathways that these plants initiate, since this provided insight into their capacity to impact programmed cell death. Additionally, this investigation explored the anti-hemolytic characteristics of the studied plants, discussing their possible contribution to reducing hemolysis, a disorder linked to a number of conditions. By conducting both *invitro* & *invivo*studies to assess the individual & combined effects of these three natural products on cytotoxic, anti-hemolytic, hepatoprotective and anti-oxidant homeostasis, this research study seeks to close this critical knowledge gap.

METHODOLOGY

For this current study, medicinal plants were obtained from the different regions of Faisalabad. Plants were taxonomically confirmed and identified from laboratory. Plant materials were used for sample preparation. After sample preparation plant materials were used for the detection of their phytochemical profiling, hepatoprotective, cytotoxic, anti-hemolytic, and anti-oxidant activity.

Based on the long therapeutic properties, *Nigella sativa, Oleaeuropea*and *Allium sativum* were selected to screen their cytotoxic, anti-hemolytic, hepatoprotective & anti-oxidant activities. The details of these plants are listed below in the table.

Table 1: Selected medicinal plants for present study

Plant	Common name	Family	Part used
Allium Sativum	Garlic	Liliaceae	Bulb
Nigella sativa	Black seed	Rananculaceae	Seeds
Oleaeuropea	Olive	Oleae	Fruit

Qualitative Phytochemical Analysis

Through qualitative analyses, the presences of several phytochemical components in the hydroethanolic extracts were identified. Alkaloids, flavonoids, terpenoids, saponins, phenolic compounds, glycosides, and other secondary metabolites were all being identified using certain

reagents. These phytochemical groups were confirmed by the colour alterations and precipitation reactions.

Quantitative Phytochemical studies

Total Flavonoid and phenolic content

The total numbers of phenolic and flavonoid compounds present in medicinal plants (*Nigella sativa, Oleaeuropaea*, and *Allium sativum*) were determined by Folin-Ciocalteu (FC) System. The results were reported in milligrams per gramme of gallic acid equivalent (mg GAE/g) and µgcatechin equivalent per milliliter (µg EC/mL).

HPLC for phenolic acids

HPLC is a crucial analytical method for evaluating phenolic acids, which are essential secondary metabolites in plants. A C18 column with a 5 µm film thickness and a 30°C temperature were used for separation and identification of phenolic acid components. A sophisticated HPLC system were used. UV spectra were taken at 275 nm to identify phytochemical compounds.

Trace elements (heavy metals) detection

The AOAC (2000) recommends a two-phase procedure for detecting trace elements in medicinal plants. The first phase involves digesting the sample with nitric-perchloric acid, boiling it, and then adding distilled water. The solution is then filtered using filter paper and distilled water. The atomic absorption spectrometer (AAS) is used to investigate the concentration of heavy metals like Copper, iron, cadmium, lead, manganese, cobalt, zinc, nickel, and Mn.

***In vitro* experiment design**

One of the most important aspects of this research is the in vitro experiment design, which adopts a comprehensive strategy to determine the free radicle scavenging activity and gauge the safety of the botanicals. This section outlines the exact methods used to carry out the *in vitro* research, including hemolytic activity, tests for mutagenicity, DNA damage prevention and anti-oxidant capacity.

Hemolytic activity Assay

The hemolytic activity assay involves preparing a suspension of erythrocytes from heparinized human platelets, a crucial part of hemostasis. The platelets are mixed with bird droppings and re-suspended in a solution of saline and isotonic phosphate (PBS) to maintain cellular integrity. The attenuated cell probe (PBS) is stored in ice water, and Triton X-100 is used as the control. The extract is mixed with the platelet suspension & incubated for 35 minutes at 37°C. The supernatant is collected and analysed, with absorption measured at 576 nm in ELISA plate pellets. The study is multiplied and kpe SE is determined using the percentage hemolysis. The results are then used to calculate the kpe SE using the % hemolysis formula.

DNA Damage Prevention Test

The DNA damage protection assay is adapted from Tian and Hua's (2005) method, with modifications for this study. Calf thymus DNA is treated with Fenton reagent, ferrous sulphate, and medicinal plant extracts. The DNA's DNA damage protection capacity is evaluated using agarose gel electrophoresis. The reaction mixtures are incubated for 60 minutes at 37°C, & then added with Bromophenol blue as loading dye. The agarose gel is electrophoresed at 85°C for 45 minutes, stained with ethidium bromide, and documented using the SyngeneGeneGenius Gel Light Imaging System.

Ames test

The Ames test, a liquid culture-based fluctuation test, is used to examine the mutagenic properties of entities. The two major strains of bacteria, S. typhimurium TA98 and TA100, were kept on nutrient agar and cultured for 18 to 24 hours at 37°C prior to the test. Chemical components include

bromocresol purple, Davis-Mingioli salt, D-biotin, D-glucose, and L-histidine. The herbal extract was extracted using a methanol/chloroform solution and reconstituted in DMSO. The reagent mixture were prepared and inoculated with S. typhimurium test strains overnight. Positive wells were scored as yellow or turbid, while negative wells were scored as purple. To be mutagenic, the number of positive wells in the herbal extract plate must be larger than the number in the background plate.

DPPH Radical Scavenging Activity

Using the well-established approach described by Ho et al. (2010), a thorough evaluation of DPPH radical scavenging activity, a key measure of antioxidant capability, was conducted. The total number (10 µL) of test cases in methanol, resulted in the development of concentrations of 5, 10, 50 and 100 µg / ml, separately, in each reaction the reaction were mixed with 200 µl of 0.1 mM DPPH ethanol arrangement and 90 µl of 50 mMTrisHCl incubator (pH 7.4). Methanol (10 µl) alone was used as a control for this study. After hatching at room temperature for 30 min, the decrease in DPPH -free revolutions were estimated by studying the absorption force at 517 nm. (+) Catechin, a remarkable cell enhancer, was used as a positive control. Three reproductions were made for each test.

***In-vivo* experimental design**

Animal model selection and maintenance

Swiss albino mice, 5 to 6 weeks old, weighing 28.2 g to 39 g, were collected from the institutional animal house of Government College University in Faisalabad. To ensure the welfare and gentle treatment of the animals, ethical principles and institutional norms must be strictly followed. Mice were housed for a week under normal conditions at temperature of 21-25^{o}C before the experiment.

Experimental groups

The symphonic structures of the experimental groups were designed to investigate the complex potential of *Nigella sativa*, *Oleaeuropea*, and *Allium sativum*, both singly and in combination.

According to the researchers, more than thousand chemicals and medicines have been reported to cause hepatotoxicity. In all animal groups CCl4 was used to persuade hepatic injury. Liquid paraffin was used for thinning of CCl4, given intra-peritoneal. The following groups were included in the design:

Group I

It was named “Control animals (Normal) group”. The number of mice in this group were 6. For this group carboxymethylcellulose (1mL/kg of 1% w/v, Per Oral) was given for 21 days.

Group II

The number of mice in this group were also 6. It was named “Intoxicated Controlgroup”.No further treatment was given to this group after CCl4 intoxication (CCl41mL/kg, I/P).

Group III:

The number of mice in this group were also 6. This group was named “Standard drug group” because standard drug was given to this group (Silymarin 200 mg/kg, Per Oral) for 21 days.

Group IV

The number of mice in this group were also 6. For this group Extract 1 (Nigella sativa 200 mg/kg, Per Oral) was given for 21 days.

Group V

The number of mice in this group were also 6. For this group Extract 2 (Oleaeuropea200 mg/kg, Per Oral) was given for 21 days.

Group VI

The number of mice in this group were also 6. For this group Extract 3 (Allium sativum200 mg/kg, Per Oral) was given for 21 days.

Group VII

The number of mice in this group were also 6. For this group Combination of all extracts (*Nigella sativa*, *Oleaeuropea*, and *Allium sativum*100mg/kg, Per Oral) was given for 21 days.

Based on consequences of previous studies on mice treatment plan was adjusted for 21 days and Standard doses of Silymarin and extract was given orally daily for 3 weeks to respective groups.
Total Oxidant Status (TOS) and Serum Total Antioxidant Status (TAS) strains & status were being identified. It is important to carefully assess the resonance of liver enzymes such AST,ALT, ALP, and total bilirubin. Total protein, albumin, & globulin vibrated in unison. The subtle measurements wereinclude urea, creatinine, uric acid, HDL, LDL, triglycerides, cholesterol, and triglycerides, each carefully carried out to understand the synergistic effects on renal function.
The focal organs, the liver, intestines, and kidneys, were removed and stored in 10% buffered formalin. The splendour of hepatic lobule alignment, the complexities of lipid alterations, and the finesse of cell infiltration revealed under a microscope (Olympus CX23), bringing this harmonic symphony of investigation to a close.

RESULTS

Study results have shown that the combination of *Nigella sativa*, *Oleaeuropea*, and *Allium sativum* have synergistic effects in addition to their separate cyto-protective, anti-hemolytic, hepatoprotective and antioxidant capabilities. Synergy occurs when the combined effects of the chemicals from these plants improve their overall effectiveness in preventing liver damage and oxidative stress. In comparison to their solo effects, this synergistic impact may have stronger anti-hemolytic, antioxidant and hepatoprotective effects.

Qualitative Phytochemical analysis

Three plants' hydroethanolic extract were subjected to an initial qualitative phytochemical screening to identify bioactive components. By using different test with their reagent Alkaloids, flavonoids, tannins, phenols, steroids, glycosides, terpenoids, and saponins were found in the sampleswhich are described in the following table (Table 2).

Table 2: Qualitative analysis of hydroethanolic extract of *Nigella stiva, Allium sativum, Oleaeuropea*

Phytochemical Class	Indication	*Nigella sativa*seed extract	*Oleaeuropea*fruit extract	*Allium sativum*bulb extract
Flavonoids	Red or pink color/	+	+	+
Alkaloids	Reddish brown ppt's	+	+	+
Glycosides	Brown color ring	-	+	-
Terpenoids	Reddish brown color	+	+	+
Diterpenes	emerald, green color	-	+	+
Tannins	Black / green black color appear	-	+	-
Saponins	Persistent layer of froth	+		+
Phenols	Red/green /blue color.	+	+	+
Amino acids		+	+	+
Proteins	Blue color	+	+	+
Carbohydrates	Brick red / red and orange ppt's	+	+	+
Starch	blue-black speck	_	_	_

(-) indicate the absence, (+) Indicate weeresenceof phytochemicals.

Quantitative Phytochemical studies

Total flavonoids and phenolic contents

The extracts' total flavonoid content (TFC) was calculated using the catechin linear regression curve and expressed as µg of catechin equivalents (CE) per millilitre of plant extract. Total flavonoids are found at levels ranging from 1.525 to 3.84 (mg / g CE). The highest flavonoid concentration (3.84µg / mL EC) was found in *Nigella sativa*, while the lowest flavonoid content (1.525µg / mL EC) was found in *Allium sativum*, where *Oleaeuropea*contained (3.401µg / mL EC) are expressed in following table.

Table 3: Total flavonoid and phenolic content in selected medicinal plants Nigella sativa, oleaeuropea and Allium sativum

Plant	TFC in sample ug CE/mL	Final TPC mg GAE/mL
Allium Sativum	470.8823529	594
Nigella sativa	1129.411765	1656
Oleaeuropea	1000.294118	1470.5

High Performance Liquid Chromatography (HPLC)

The phytochemicals found in the hydroethanolic extracts of particular medicinal plants were identified using HPLC-UV. Following the protocol's optimisation for the analysis of phenolic compounds, a run time of 20 minutes and a wavelength of 227 nm were employed to improve the chromatogram's resolution for the chemical components found in the extracts. The findings demonstrated that the chosen medicinal plants included a variety of phenolic chemicals, some of which may have potent antioxidant properties.

Table 4: Compounds Identified by HPLC in Selected Active Plants*Nigella sativa, oleaeuropea and Allium sativum*

Standard	Retension time	*Nigella sativa* seed extract	*Oleaeuropea*fruit extract	*Allium sativum*bulb extract
Chlorogenic Acid	2.88	+	+	+
p-cumaric Acid	3.166	+	+	+
Gallic Acid	3.342	+	+	+
Caffeic Acid	7.494	+	+	+
Vinilic Acid	7.687	+	+	+
Kaemphferol	11.696	+	-	+
Sinapic Acid	12.237	+	+	+
Ferulic Acid	12.46	+	+	+
Salicylic Acid	15.296	-	+	+
Coumarin	16.085	+	-	-
Quercetin	16.954	+	+	+
Benzoic Acid	18.306	-	-	+
Rutin	23.989	+	+	+

Mineral contents of selected medicinal Plants

The study analyzed the mineral content of medicinal plants, including essential and toxic elements such as Magnesium (Mg), Zinc (Zn), Iron (Fe), Calcium (Ca), Arsenic (As), Cupper (Cu), Iron (Fe), Cadmium (Cd), Lead (Pb), and Mercury (Hg).Samples were digested using AOAC's nitric-perchloric acid method, and mineral contents were determined using an atomic absorption spectrometer. Heavy metal levels were expressed as mean concentrations in the following table.

Table 5: concentration of heavy metal in (mg/kg) selected medicinal plants Nigella sativa, oleaeuropea and Allium sativum

Heavy Metals	Nigella sativa	Oleaeuropea	Allium sativum
Iron (Fe)	78.5±5.9	111.70±9.80	5.61±0.29
Zinc (Zn)	27.3±1.5	2.23±0.06	10.47±0.003
Calcium (Ca)	1095.73±25.65	1,291 ± 75	34
Mercury (Hg)	-	-	-0.0043±0.000
Copper (Cu)	3.06±0.26	6.40±0.09	0.68±0.000
Manganese (Mn)	8.47±0.22	6.40±0.05	0.25±0.001
Cobalt (Co)	10.1±0.3	0.09±0.05	0.78±0.003
Cromium (Cr)	-	0.68±0.02	0.14±0.004
Arsenic (As)	-	0.03±0.01	-
Lead (Pb)	-	0.83±0.02	0.33±0.000
Cadmium (Cd)	-	0.05±0.02	0.18±0.000
Nickel (Ni)	-	0.31±0.02	0.013±0.001

Mutagencity Test by Ames Assay

The Ames test was used to assess the mutagenicity of medicinal plants using liquid culture. Salmonelatyphimurium TA98 and TA100 were used as bacterial mutant strains. The test revealed that all plants were possessing both mutagenic and non-mutagenic. The Fold rule was used to determine mutagenicity, with the number of positive wells in the test plate being twice as high as the number in the background plate.

Table 6: Mutagenic activity of selected medicinal plants *Nigella sativa, oleaeuropea and Allium sativum*against*S. typhi* TA98 and TA100

Plant	No. of positive wells/ *S. typhimurium*TA98	Results	total no. of wells *S. typhimurium*TA100	Results
Background	3 / 96		4/ 96	
Standard	93 / 96	Mutagenic +	75 / 96	Mutagenic +
Allium Sativum	1/ 96	Non-mutagenic	38/ 96	Mutagenic +
Nigella sativa	53 / 96	Mutagenic +	11 / 96	Non-mutagenic -
Oleaeuropea	30/96	Non-mutagenic -	44 /96	Mutagenic +

Compared to the control, there is a substantial increase in the number of positive wells ($p<0.05$) indicated by "+"; no discernible influence is indicated by "-".

Hemolytic Activity

The safety of medicinal plants is crucial in drug development, and evaluating their toxicity is essential before starting animal trials. A hemolytic assay using human red blood cells (RBCs) was used to explore the cytotoxic potential of selected plants. The results showed that plant hemolysis rates were significantly lower than triton X-100 (positive control). Low hemolytic activity indicates the presence of small or non-toxins in human erythrocytes. Achyranthesaspera'shemolytic activity against human erythrocytes is poor, while Lantana camara's aqueous form and solvent components have considerable hemolytic action. Allium stracheyi extracts have strong hemolytic activity against human red blood cells.

Table 7: Hemolytic activity of selected medicinal plants (*Nigella sativa, oleaeuropea and Allium sativum*) in different combinations

Plant	Absorption	Negative control	Triton-X	% Hemolysis
Allium Sativum	0.075	0.07	0.418	1.19617225
Nigella sativa	0.082	0.07	0.418	2.8708134
Oleaeuropea	0.148	0.07	0.418	18.6602871

Anti-oxidant potential

Antioxidant phytochemicals prevent substrate oxidation and detoxify free radicals, preventing oxidative stress and causing metabolic and health problems. Herbal antioxidants like phenolics, carotenoids, flavonoids, vitamins, and food supplements help improve antioxidant status. A study evaluated the antioxidant potential of selected medicinal plants' hydroethanolic extracts, revealing significant antioxidant activities ($p<0.05$).

Table 8: Antioxidant Activities of selected medicinal plants (*Nigella sativa, oleaeuropea and Allium sativum*)

Plant	Absorption	Blank absorbance	% DPPH inhibition
Nigella sativa	0.361	0.575	37.2173913
Oleaeuropea	0.469	0.575	18.43478261
Allium sativum	0.502	0.575	12.69565217

Animal model

First of all, mice were acclimatized for 4 days at temperature 21-25. They were provided with feed, pure water ad libitum. Their condition and body weight was checked regularly. After 4 days, mice were treated with CCL4 according to their body weight to induce hepatotoxicity. Befor hepatotoxicity the level of all liver enzymes including ALT, AST, ASP, SGPT, SGOT and bilirubin were within the normal range. After Hepatoxicity, all these markers were elevated. After that dosage were prepared for the treatment of Hepatoxicity by using the hydroethanolic plant extract of *Nigella sativa, Oleaeuropea* and *Allium sativum*. DSMO and normal saline were added to extract for preparation. The treatment plan was of 21 days. Extract dosage was given according to the body weight.

For group 1, only carboxy methyl cellulose was given for 21 days. For group 2, no treatment was given after CCL4 intoxication. For group 3, silymarin (200mg/kg body weight) was given. For group 4 5 and , Nigella sativa, Oleaeuropea and Allium sativum extracts (200mg/kg body weight) were given respectively. The last group (7) was treated with combined extract of all these three plants (*Nigella sativa, Oleaeuropea* and *Allium sativum*) was given (100mg/kg body weight).

After the experiment, the rats were put to sleep, and blood samples were taken from their hearts. Cervical dislocation was used to scarify anaesthetized animals during blood collection. After the belly was opened, the organs—the heart, liver, kidney, and testes—were quickly removed, weighed, and preserved in saline. For histological investigations, a portion of liver was preserved in 10% buffered formalin after being cleaned in sterile saline. To prepare liver tissue homogenate for the measurement of interleukin-6, lipid peroxide, and antioxidant enzymes, a portion of the liver was stored at an ice-cold temperature.

After 21 days treatment to evaluate the hepatoprotective activity different protein level and liver enzymes in serum samples, tissues specimens and other body fluid were evaluated. The results have demonstrated that these plants extracts (*Nigella sativa, oleaeuropea* and *Allium sativum*) have significant potential for the regulation of live enzymes in CCL4 intoxicated mice. The elevated Serum alanine aminotransferase (ALT) level in mice was returned to normal on treatment with hydroethanolic plant extracts. For AST, ALP, SGPT, SGOT significant reduction was noted. Besides the hepatoprotective activity, anti-oxidant, renal protective, anti-hemolytic activities were alsio

observed.

DISCUSSION

Medicinal plants have been utilised as traditional medicines for treating a wide range of illnesses since ancient times. Variousresearch show that certain potential phytochemicals can be created as substitutes for manufactured medications to treat various health issues (AlSheikh et al., 2020).Numerous plants have been shown to exhibit significant hepatoprotective properties among these. It is still necessary to look for novel, cutting-edge medications to treat liver illnesses. Liver disease prevalence is comparatively increasing, and considerable rates of death and morbidity are associated with it (Huang et al., 2023). The current study was focused at studying hepatoprotective, anti-hemolytic, cytotoxic and anti-oxidant activity of *Nigella sativa, Oleaeuropea* and *Allium sativum*to CCL4 induced hepatotoxicity in mice.

Plant extracts lessen serum enzyme disruptions in the bloodstream and enhance liver function. Strong radicals and hepatoprotective compounds can be found in silymarin, an essential antioxidant (Karimi et al., 2011).Treatment with silymarin showed the low cost of inflammation, mild fibrosis, and necrosis. It served as a positive control with a liver-protective impact on the hepatotoxicity of CCl4 and was employed as an indicator of SD in the current investigation.In the current study, a dose of 200 mg/kg per selected plant *Nigella sativa, Oleaeuropea* and *Allium sativum*given orally to rats showed no adverse effects. Daily monitoring of a dose (200mg/kg) of a crude extract for 21 days had no harmful effects. All plants used in our study were eligible for further investigation into their hepatoprotective and hepatotoxic effects of CCl_4. The hepatoprotective function of the selected plant was evaluated by ALT, AST, ALP, TB and histology compared. A significantly reduced level of hepatotoxicity was improved by the combined plant treatment ($p<0.01$) (100 mg/kg) for all selected plants.

Because of the plant's high antioxidant and anti-inflammatory action, it protects hepatocytes from cell damage caused by CCl_4 stress and lipid peroxidation (Callej*a et al*., 2013), which is characterized by lower biochemical and serum liver levels than a single control agent.Three plans *Nigella sativa, Oleaeuropea* and *Allium sativum* demonstrated superior protective effects in mice against CCl_4-induced toxicity, and our work was another attempt to investigate the activity of antioxidants cytotoxic, anti-hemolyticand plant components with hepatoprotective properties in the mice. Regarding synergetic treatment combine plants showed more significant results as compared to single plant effects. After treatment with combined plants, it was more significant ($p <0.01$).

CONCLUSION

The current research work was done to discover the selected medicinal plants *Nigella sativa, Oleaeuropea* and *Allium sativum*forhepatoprotective, cytotoxic, anti-hemolytic& antioxidant potentials*in vitro* &*in vivo* model.the phytochemical profiling was done by qualitative tests to investigate the potential phytochemical constituents of plants. The in vitro study investigate the total phenolic and flavonoid content by performing HPLC and FTIR. Ames test, DNA demage prevention, DPPH, hydrogen peroxide assay, reducing power assay were performed. Investigations conducted in vivo using albino mice as a model animal to investigate the potential of plant extracts for hepatic restoration Along with CCl4 intoxication, 200 mg/kg of each plant extract & 100 mg/kg of the synergetic were given. At the conclusion of the study, baseline blood parameters including haematological, oxidative stress indicators, LFTs, RFTs, lipid profile, serum protein, and serum electrolytes were examined.The results indicated that certain therapeutic plants have a considerable ($p<0.05$) capacity for repair. It is possible to draw the conclusion that certain medicinal plants have a major therapeutic response to treat medical issues, especially liver ailments.

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