Research Article

Antioxidant and protective effect of "*Artemesia absinthium*" and "*Nigella sativa*" on Albino mice mode of hepatic injury

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ABSTRACT

Background: Ethanoic plant extract of *N.sativa* and *A.absinthium* has compounds that act as anti-inflammatory, cardio protective, anticancer, and antioxidant. The flavonoids and reducing power in these plants are used to treat liver injury. In this research, we explored the protective effect of these plants on APAP-induced liver injury.

Methodology: Albino mice were treated with extract of both plants separately and then used in combined form for 14 days, the results show the protection against injury induced in the 23 liver by APAP (220 mg/kg). *In vitro* study, the plants extract shows phytochemical compounds by pre-treatment assay, reducing assay, and DPPH assay. In this study, albinos were categorized into 5 groups, group I was the control group that received only distilled water, and group II received 220 mg/kg Acetaminophen by intraperitoneal injection. Group III received 350 mg/kg ethanolic extract of *N.sativa* and received 220 mg/kg acetaminophen. Group IV received 350 mg/kg ethanolic extract of *Artemisia absinthium* and received 220 mg/kg acetaminophen. Group V received both 500 mg/kg ethanolic extract of *N.sativa* and *A.absinthium* and received 220 mg/kg acetaminophen.

Results: The results indicate that the extract of *N.sativa* and *A.absinthium* decreased the AST and ALT level in serum in exposed APAP mice. However, the combined form of both extracts decreased the LFTs in blood serum.

Conclusion: This study proved that the plant extract is used against APAP hepatic injury and it is also associated with oxidative stress in the body. And the combined extract maybe use as a therapeutic agent against hepatitis.

Keywords: Antioxidant activity, TPC, TFC, DPPH, *Artemesia absinthium*, *Nigella sativa*, Hepatocellular carcinoma, Antibacterial, Anticancer activity.

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INTRODUCTION

Acute hepatitis and hepatocellular carcinoma are severe medical conditions due to the release of inflammatory cytokines. (IL-6, TNF-α, and INF-γ), elevated ALT /AST, cell regulation stop that progress to fibrosis cirrhosis and cancer [1]. Viruses like HCV and HBV, toxins, autoimmune alcohol, metabolic disorders like Williamsons, obesity, diabetes, drugs are the main risk factor of liver pathogenicity [2]. Xenobiotics compound absorbed into the gut and then transported to the liver for metabolism. During metabolisation, some toxic intermediates generated and damage the liver parenchymal cells and hepatocyte cells [3].

Due to mortality, Hepatocellular carcinoma ranks second among all cancers. Pakistan has the highest prevalence rate of hepatitis C and it's known as a risk factor of HCC [4]. HCC is treated with immune suppressive therapies which are associated with sides effects like osteoporosis and hyperglycaemia [5].

The liver involves in different functions like metabolic, detoxifications, chemical transformation, excretory, and supporting other organs [6]. Traditional medicines use as protection against cancer. Green tea extracts have a hepato protective compound that uses against APAP to induce hepatotoxicity [7]. *Ficus carica* is commonly known as anjeer, used for medical purposes in India. Another plant *Bauhinia purpurea* L., commonly known as kachnar, is used to treat stomach ulcers, ulcer wounds, fever, diarrhoea, and swelling [8].

METHODOLOGY

Chemicals

Chemicals were purchased from Sigma-Aldrich except for Folin-Cioacalteu's. Disodium phosphate buffer solution purchased from Fisher Scientific.

Collection of plant sample

N.sativa was collected from the market that was available in herbal shops. Fully maturated *A. absinthium* plant with their roots collected from northern areas of Pakistan. Collected specimens were dried at room temperature and stored for further use.

Preparation of plant extracts

The leaves and seeds of *A.absinthium* and *N.sativa* were washed carefully with distilled water and crushed into fine powder. 100 g of powder sample soaked in ethanol for 48 h at room temperature. The solid particles were removed from the solvent with the help of fine cloth and concentrated solvent at 50-600°C by using a rotatory evaporator. The extract was stored at -20°C for further use.

In vitro studies

Total phenolic assay: 70 mg of the dry mass of plant extract was mixed with 0.7 ml of Folin-ciocalteu reagent and 7.5 deionized water. Check absorbance at 755 nm at spectrophotometer. Results were expressed as mg/ml gallic acid equivalents.

Total flavonoids assay: Total flavonoids detection by Chin and Li

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method. The solution absorbance was measured at 510 nm in a spectrophotometer. The results were expressed as catechin equivalents per dry mass.

DPPH assay

2-2-diphenyl-1-picrylhydrazyl radical-scavenging activity was determined by Brand-Williamson's method. The absorbance was measured at 750 nm, all determinations were analysed in a triplicate manner.

Experimental animals

The male Swiss albino mice were used for this study and their weight was 28-32 g. Animals were housed in cages and maintained temp at 25 ± 2°C with light and dark cycle 12/12. These were provided with better nutrition.

Experimental protocols

All the animals were categorized into 5 groups, group I was the control group that received only distilled water for 14 days and group II receiving 220 mg/kg acetaminophen by intraperitoneal injection. Group III received 350 mg/kg ethanolic extract of *N.sativa* for 14 days and received 220/mg acetaminophen and group IV received 350 mg/kg ethanolic extract of *Artemisia absinthium* for 14 days and received 220/mg acetaminophen. Group V received both 500 mg/kg ethanolic extract of N.sativa and *A. absinthium* for 14 days and received 220/mg acetaminophen. On the 15th day, all animals received acetaminophen via IP injection with the respective dose mentioned above except the control group.

All animals were anesthetized with light chloroform and sacrificed by cervical decapitation on the 15th day after the final dose. The blood samples were collected in gel vials for serum preparation. Hepatotoxicity was indicated by a significant elevation in ALT and AST in acetaminophen mice as compared to controls.

Biochemical investigation

After collection of blood, serum was separated by using a centrifugation machine at 4000 rpm for 15 minutes. ALT, AST, Total bilirubin, and alkaline phosphatase enzyme were evaluated by adopting kit procedures at the LTM-9200 chemistry analyser [9].

Statistical analysis

The statistical analysis was performed by Graph Pad Prism software 9.3.1.471. The data were represented as mean ± SD and the data were subjected to one-way ANOVA

RESULTS

A.absinthium exhibits the total phenolic compound 5.79 ± 1.20 mg gallic acid equivalent /g of dry plant extract and total flavonoid content 132.55 ± 2.29 mg catechin equivalent. *N.sativa* exhibits the total phenolic compound 7.46 ± 2.49 mg gallic acid equivalent/g of dry plant extract and total flavonoid content 47.89 ± 5.14 mg catechin equivalent. The *A. absinthium* and *N.sativa* have DPPH radical scavenging property and 25 ug/ml exhibit 35.17 ± 1.19 and 24.69 ± 2.17 respectively (Table 1 and Figure 1)

The reducing power of tested samples is directly proportional to sample concentration. The reducing power at 200 ug/ml was 0.468 for *A. absinthium* and 0.637 for *N.sativa*.

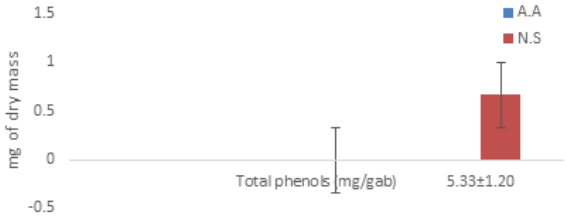

Figure 1: The total phenols, flavonoids content, and DPPH radical scavenging property of *A. absinthium* and *N.sativa*.

Pre phytochemical analysis

In this study, dried extraction was obtained from both species. Pre-Phytochemical analysis shows the presence of saponins, glycosides, diterpenes, glycosides, and steroids in N.sativa and *A. absinthium* in Figure 2.

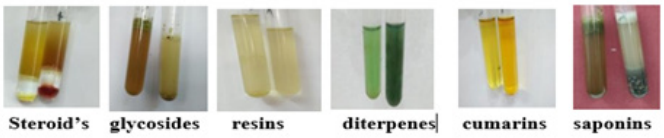

Figure 2: Pre phytochemical analysis of *N.sativa* and *A. absinthium*.

LIVER INJURY

500 mg/kg dose of APAP given to mice and compare with control group mice. As shown in the picture the challenged Albino mice's large liver lobe colour changed into a pick, the phenotypically changes in the liver that tells us about the liver injury (Figure 3).

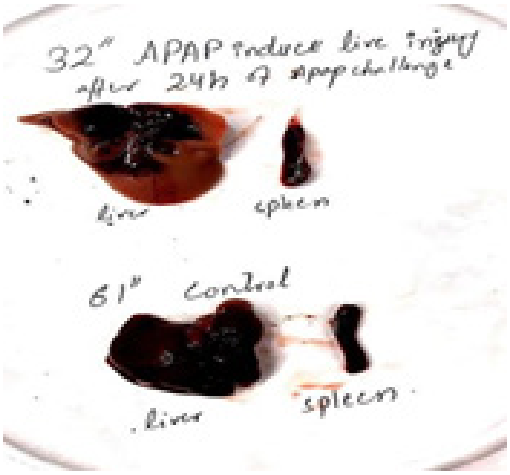

Figure 3: Liver injury of APAP mice as compared to control mice.

Table 1: The total phenols, flavonoids content, and DPPH radical scavenging property of *A. absinthium* and *N.sativa*.

Content	% Yield	Total phenols (mg/gab)	Total flavonoids (mg/gac)	Total flavonoids mg/g of extract	Total phenolic mg/g of extract	DPPH radical scavenging at 25 ug/ml extract
A. Absinthium	13.02%	5.33 ± 1.20	135.55 ± 2.29	0.67	0.1	35.17 ± 1.19
N.sativa	10.66%	7.46 ± 2.49	49.15 ± 4.83	0.16	0.12	24.69 ± 2.17

Note: [ab] Phenols; [ac] Flavonoids

The effect of plant extracts, acetaminophen caused liver damage in mice as evidenced by changes in hepatic enzymes LFTS. The normal ALT value 54 IU/L increases 186 IU/L, AST value 144 IU/l was elevated to 256 IU/L, and ALP level 205 IU/L increases 436 IU/L by APAP intoxication. The *A. absinthium* and *N.sativa* 350 mg /kg the ALT mean value observed 51.78 IU/L and 48.27 IU/L Respectively. Combined plant extract group ALT and AST mean value observed as 45.79 IU/L and 44.1 IU/L. Bilirubin has no minor changes and the ALP mean value was observed as 178.5 IU/L (Figures 4-7).

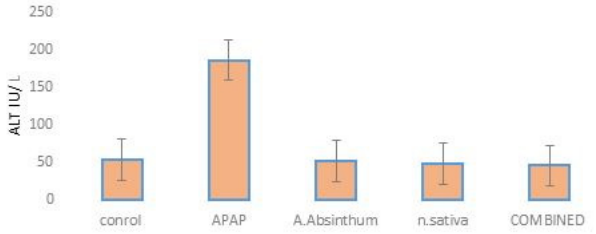

Figure 4: Aqueous ethanolic extract of plants *A. absinthium* and N. sativa inhibited ALT (levels in serum).

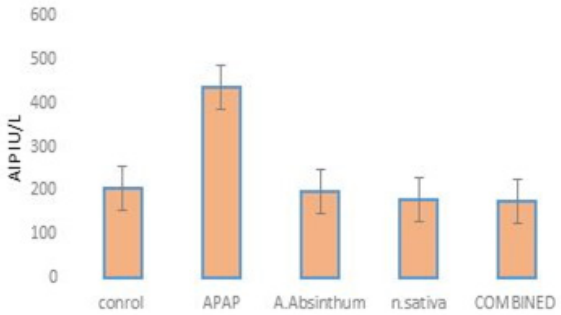

Figure 5: Aqueous ethanolic extract of plants *A. absinthium* and N. sativa inhibited AST (levels in serum).

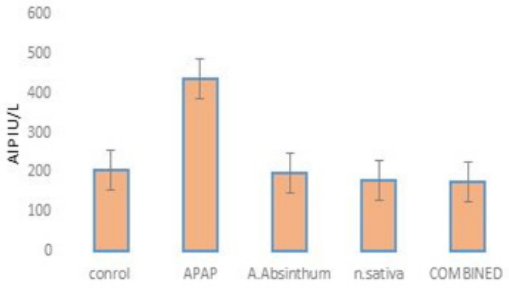

Figure 6: Aqueous ethanolic extract of plants *A.absinthium* and *N.sativa* inhibited AIP (levels in serum).

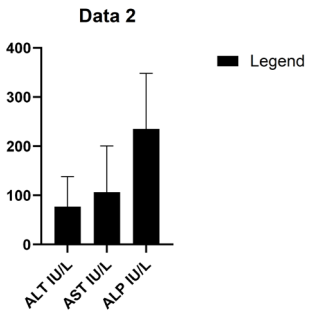

Figure 7: Aqueous ethanolic extract of plants *A. absinthium* and N. sativa inhibited LFT's (levels in serum).

DISCUSSION

The process of the discovery of drugs is becoming more complex and more critical. Especially, the screening of the plants having anticancer activity has been increased since the few past decades. The approach for the screening and identification of the medicinal plan is based on ecological, ethno pharmacological, and chemo systemic information. Some of the institute research on hepatitis is working for the screening of the compounds having anticancer activity from the medicinal plants.

There exist many approaches for the researchers, but the more convenient method is the APAP which is usually used commonly in health labs to treat the liver injury *in vivo*. APAP model provides many opportunities for the researchers to investigate the mechanism of treatment of hepatitis disease. So, liver injury including cirrhosis even hepatocellular carcinoma can be treated by adopting the APAP approach.

There are many plants such as *A. absinthium* and *N.sativa* that have been reported, to have protective effects against acetaminophen-induced liver toxicity in mice. Another plant is known as ''Sal" which alleviates oxidative damage in the livers of albino mice. All these findings suggest that medicinal plants play important role in the treatment of hepatocellular carcinoma [10, 11].

The present study tells that the combined extract of both plants reduces the ALT, AST, and acts as an anti-inflammatory agent against PARP-induced liver injury. After IP Injection, PARP absorb in the liver and binds with glycoproteins that are mannose-rich glycoproteins and they present on sinusoidal endothelial cells of the liver and activates the CD+ T cells and then T cells secrete interleukins, cytokines, and adipokines. When infection occurs the elevation of transferases occurs in the blood. In this study, the results show that the extract of both plants suppressed the release of cytokines in the liver, and the inflammatory reaction inhibited by extract in APAP induced liver injury [12].

Plants have natural products that play role in human health and other illness because they have bioactive compounds. The phenolic, flavonoids, and carbohydrates are bioactive compounds and also affect cells [13]. In this study, both plants have these compounds that act as anti cancerous and protective properties.

ROS is the major product of the metabolic process; it causes oxidative stress in cells and leads to liver injury. Natural compounds have antioxidant properties and are used in the treatment of liver injury [14]. In this study, a DPPH assay was used to access the free radicals potentials in both plants. The antioxidant activity of natural products is performed by DPPH scavenging activity and power reduction.

PARP-induced liver injury is the common leading hepatic injury. Hepatocellular injury due to excessive level of PARP that increases the level of AST and ALT in blood. Generally, ALT and AST enzymes are used as biomarkers of liver disorders [15]. In our study significantly higher level of aminotransferases is due to higher PARP induction in liver injury. *A. absinthium* and *N.sativa* singly and combined reduced the ALT and AST level in serum and also have hepato protection potential.

In conclusion, the extract of *Artemisia absinthium* and *Nigella sativa* plant was used for the treatment of liver injury. These plant extracts have flavonoids and phenolic compounds that act as antioxidant properties as well as reduce the AST and ALT level against PARP-induced liver injury. Maybe it is associated with suppressing oxidative stress. The results show that the extract of *Artemisia absinthium* and *Nigella sativa* has potential against hepatitis and in the future its use as therapeutic medicine against liver injury.

CONCLUSION

In our study significantly higher level of aminotransferases is due to higher PARP induction in liver injury. *A. absinthium* and *N.sativa* singly and combined reduced the ALT and AST level in serum and also have hepato protection potential. We found that the DPPH assay has a strong negative correlation with the HepG2 cell line which indicates that DPPH decreases cell growth through the ROS scavenging mechanism. This study proved that the plant extract is used against APAP hepatic injury and it is also associated with oxidative stress in the body. The extract of *Artemisia absinthium* and *Nigella sativa* plant used for the treatment of the liver injury. These plant extracts have flavonoids and phenolic compounds that act as antioxidant properties as well as reduce the AST and ALT level against PARP-induced liver injury. Maybe it is associated with suppressing oxidative stress. The results show that the extract of *Artemisia absinthium* and *Nigella sativa* has potential against hepatitis and in the future its use as therapeutic medicine against liver injury.

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CONFLICT OF INTEREST

All authors have no conflict of interest

CONSENT FOR PUBLICATION

This study is based on research.

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AVAILABILITY OF DATA AND MATERIALS

The data associated with a paper is available, the data available on demand through email contact of co-author.

ETHICAL APPROVAL

All applicable international, national, and institutional guidelines for the care and use.

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