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TO EVALUATE THE HEPATOPROTECTIVE ACTIVITY OF *DAUCUS CAROTA* L AND *CAMELLIA SINENSIS* EXTRACT IN COMBINATION AGAINST AZATHIOPRINE-INDUCED LIVER INJURY IN RATS

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Keywords:

Hepatotoxicity, Herbal preparation, *Daucus Carota L*, *Camellia Sinensis*, Antioxidant activity

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ABSTRACT: Background: *Daucus carota L* contains a variety of carotenoids and well known for their antioxidant properties, in particular β-carotene. The antioxidant capability of polyphenols present in *Camellia sinensis* has been reported to be both chemo-protective and healing. The present work is about to evaluate the Hepatoprotective activity of *Daucus carota L* and *Camellia sinensis* extract combination against azathioprine-induced liver injury in rats as a model and determine the hematological findings, biochemical parameters and histophathological changes in liver section of rats. **Methods:** To evaluate the hepatoprotective activity of combination animals were divided into 6 groups of six rats in each group. Group I a normal control group which received normal saline on the daily basis by orally. Group II a disease group treated with drug model azathioprine 50mg/kg (w/w) i.p route to induce disease. Group III a standard drug group and the rats are treated with drug silymarin 50mg/kg (w/w) p.o Group IV test drug 1 (carrot extract) 25ml/kg body weight by p.o route. Group V test drug 2 (Green tea extract) 100mg/kg body weight by p.o route. Group VI test drugs (1+2) in combination (25ml/kg + 100mg/kg) body weight by p.o route. **Results:** Azathioprine showed pathological, hematological and biochemical findings in liver. The herbal preparation prepared showed the synergistic effect and balance the level of reactive oxygen species. **Conclusion:** To conclude, that the *Daucus carota L* and *camellia sinensis* combination exhibits hepatoprotective activity through their antioxidant, anti-inflammatory and antiapoptotic mechanisms.

INTRODUCTION: Azathioprine is among the oldest pharmacologic immunosuppressive agent. It suppresses the immune system and elevates the liver enzymes, depletion of hepatic reduced glutathione, catalase and glutathione peroxidase, accumulation of oxidized glutathione, lipid peroxides and reduction in hepatic total antioxidant activity.

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It decreases the serum total proteins and elevates the value of liver protein carbonyl content. Azathioprine converted into its active prodrug 6-mercaptopurine with the help of enzyme glutathione-S-transferase. This reaction consumes the glutathione, a mother of all antioxidants which is abundantly present in hepatocytes.

Azathioprine is also directly metabolized to form inactive metabolites with the help of enzyme xanthine oxidase i.e., 1-methyl-4-nitro-thioimidazole. Oxidation by xanthine oxidase has the potential to generate reactive oxygen species (ROS), as a result lipid peroxidation, directly inhibition nucleic acid and protein synthesis as a

result mitochondrial depletion. Mitochondrial toxins deplete the ATP level which leads to mitochondrial permeability transition. Mitochondria also play important role in cell death by apoptosis, can follow the exposure of hepatotoxins and mitochondrial transition either by apoptosis or necrosis [3].

Different herbal extracts are used from the early 4th century B.C. and primary mentioned by Theophrastus. Drug combination remedies have synergistic effects which can boom the achievement rates of drug repositioning. The identification of effective drug combinations could lead to better understanding of complex disease pathophysiology which is helpful to design the effective remedy for the disease [4].

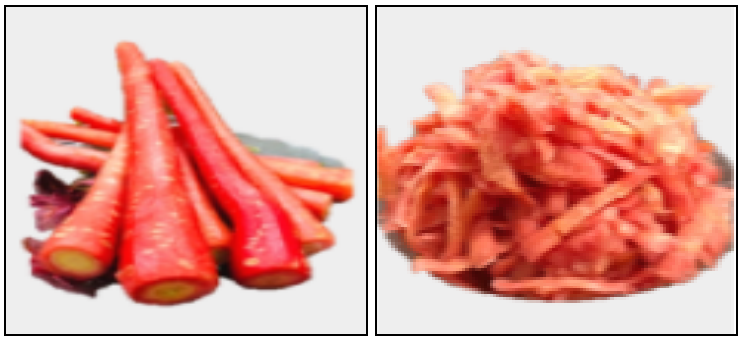
Daucus carota L and *Camellia sinensis* is a natural and excellent source of antioxidants. The herbal preparation prepared from the extracts of these drugs showed the synergistic effect through their antioxidant, anti-inflammatory and antiapoptotic mechanisms.

MATERIAL AND METHODS:

Chemicals: Azathioprine (model) 50mg/kg (w/w), *silymarin* 50mg/kg (w/w). *Daucus carota L* (Indian red carrot) fruit part and *Camellia sinensis* (Kangra green tea) air dried leaves were purchased from local market, identified and authenticated from National institute of science communication and information sources (NISCAIR). Methanol, Tween 20 (10%), Thiobarbituric acid, Phosphoric acid, n-butanol, 4% paraformaldehyde, paraffin, hematoxylineosin (H&E), All the chemicals used are analytical grade and obtained from approved vendor.

Extraction Procedure:

***Daucus carota L*:** To obtain the extract, two hundred gram fresh roots of *Daucus carota L* homogenized squeezed by means of cheesecloth without adding water into it. This freshly prepared extract was orally given according to dose described in **Table 1** by using intra-gastric catheter tube to ensure the secure and proper ingestion of the extract.



INDIAN RED CARROT

***Camellia sinensis*:** 100 g leaves of *camellia sinensis* macerated in 600 ml methanol in an Erlenmeyer flask for 72 h at 25 °C. The flush extract (Filtrate) was separated by using filter paper (Whatman No.1). The methanol from flush extract was allowed to evaporate under low pressure by using a rotary evaporator. The proportion harvest of resulting concentrated extract 11.6% (w/w). This concentrated proportion further liquefied in 10% Tween 20 and fed by orally once a day.

KANGRA GREEN TEA

Animals Procurement and Experimental Protocol: Animals (36 adult male, Wistar albino rats weighing between 150-200g) were kept in animal House Facility before one month from the study started in polypropylene cages, temperature 25±2°C, relative humidity 60±5%, standard pellet diet and water *ad libitum* to animals so that they familiar with us throughout the study. After one month, animals were divided into 6 groups, six rats in each group.

Experiment Protocol and Study Design: In experimental protocol animals were divided into 6 groups, six rats in each group. I.e., Group I a normal control group which received normal saline on the daily basis by orally. Group II a disease group treated with drug model azathioprine 50mg/kg (w/w) i.p route to induce disease. Group III a standard drug group and the rats are treated with drug silymarin 50mg/kg (w/w) p.o Group IV Test drug 1 (carrot extract) 25ml/kg body weight by p.o route. Group V test drug 2 (Green tea extract) 100mg/kg body weight by p.o route. Group VI Test drugs (1+2) in combination (25ml/kg + 100mg/kg) body weight by p.o route as in **Table 1.**

TABLE 1: ANIMALS AND EXPERIMENTAL DESIGN

S. no.	Groups	Treatment	Dose	Route of administration	Animals required
1.	I (control)	Normal saline	Daily basis	p. o	6
2.	II (Disease group)	Azathioprine	50mg/kg (w/w)	i.p	6
3.	III (Standard group)	Silymarin	50mg/kg (w/w)	p. o	6
4.	IV (Test drug 1)	Carrot extract	25ml/kg (w/w)	p. o	6
5.	V (Test drug 2)	Green tea extract	100mg/kg	p. o	6
6.	VI (Test drug 1+2 in combination)	(Carrot extract green tea extract) in combination	25ml/kg+100mg/kg	p. o	6

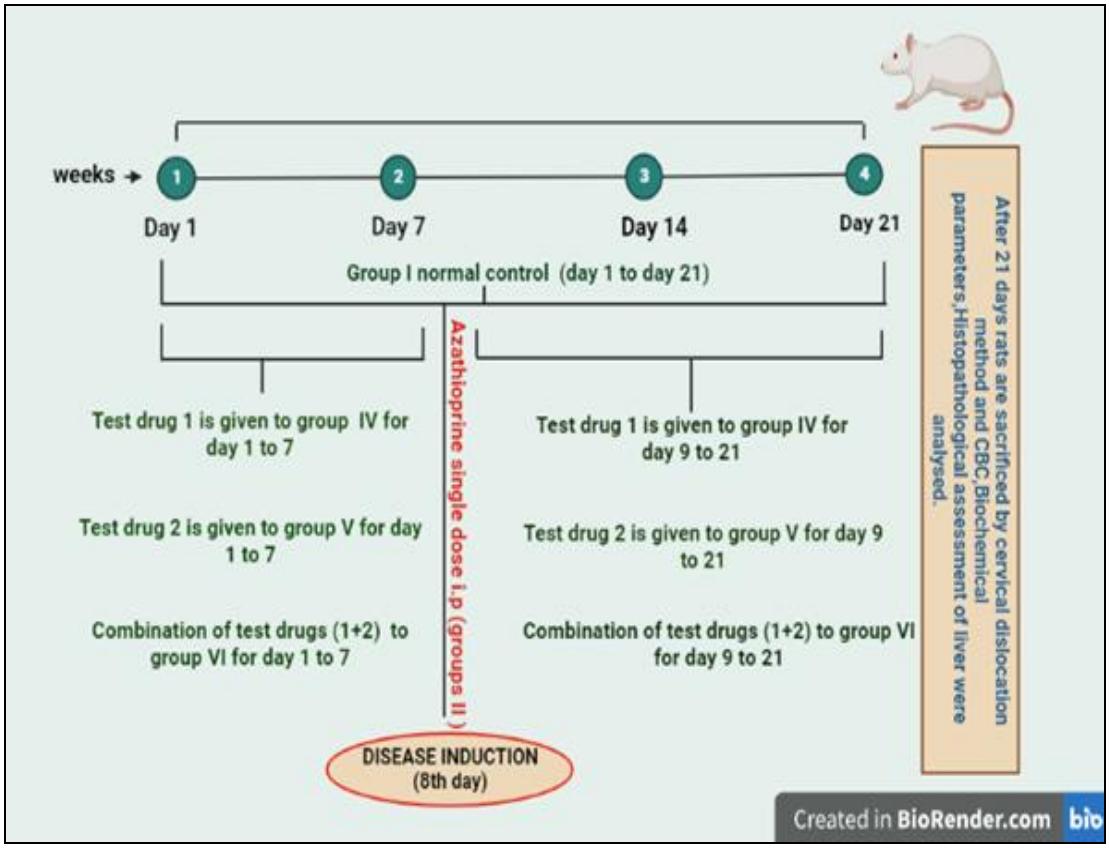


FIG. 1: TOTAL DAYS REQUIRED FOR THE STUDY WILL BE 21 DAYS. GROUP I: NORMAL SALINE FOR ALL 21 DAYS. GROUP II: AZATHIOPRINE SINGLE DOSE WAS GIVEN ON 8TH DAY. GROUP III: *SILYMARIN* 1-7 DAYS, 8TH DAY AZATHIOPRINE, 9 TO 21ST DAY *SILYMARIN*. GROUP IV: *DAUCUS CAROTA L* EXTRACT1-7TH DAY, 8TH DAY AZATHIOPRINE AND 9-21ST *DAUCUS CAROTA L EXTRACT*. GROUP V: *CAMELLIA SINENSIS* EXTRACT1-7TH DAY, 8TH DAY AZATHIOPRINE, AND 9-21TH*CAMELLIA SINENSIS* EXTRACT. GROUP VI: COMBINATION1-7TH DAY, 8TH DAY AZATHIOPRINE AND 9-21ST DAY COMBINATION WAS GIVEN.

Animals were sacrificed by giving light anaesthesia i.e ether after 8 hrs fasting from the last treatment by cervical dislocation. Blood samples are collected in ethylene diamine tetra acetic acid and liver tissue is collected in 10% formalin solution for histopathological findings.

Evaluation of Different Parameters:

Complete Blood Count: Total number of erythrocytes, total number of leukocytes, differential leukocyte count, platelet count, packed cell volume, and hemoglobin concentration were estimated by adopting standard procedures.

Blood smears were prepared as soon as possible after blood collection on a glass slide and quickly dried and stained with Giemsa and May-Grunwald stain for the differential blood count. Erythrocyte indices like mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated according to standard formulas.

Biochemical Parameters: Estimation of biochemical parameters in serum i.e the levels of alanine transaminase, aspartate transaminase, alkaline phosphate, with the help of biochemistry analyzer kit with corresponding reagents.

Histopathological Assessment of Liver: The tissue of liver fixed in 4% paraformaldehyde was embedded in paraffin, sectioned into 5 μm thickness, and stained with hematoxylineosin (H&E) for evaluation of histopathological changes. The histopathological changes of stained liver slices were observed under a bright-field microscope.

Statistical Analysis: Statistical analysis was carried out by one-way analysis of variance (ANOVA) for significant value. Data will express as standard deviation (mean±SD).

RESULTS:

TABLE 2: HEMATOLOGICAL FINDINGS AFTER 24 HRS POST TREATMENTS

Test Name	Control Group	Disease Group (Azathioprine)	Standard Group *(Silymarin)*	Test Drug 1 *(Daucus carota L)*	Test Drug 2 *(Camellia sinensis)*	Combination *(Daucus carota L+ camellia sinensis)*
RBCs (10^6/µl)	8.31±0.2	6.48±0.2[a]	7.92±0.6[b]	7.21±0.[9a,b]	7.23±0.6[b]	7.63±0.1[b]
WBCs (10^3/µl)	16.91±0.6	12.27±0.3[a]	15.79±0.5[b]	15.02±0.3[a,b]	15.6±0.2[b]	15.27±0.5[b]
Platelets (10^3/µl)	807.6±36.4	553.3±86.5[a]	725.21±11.8[a,b]	687.23±10.7[a,b]	692.25±10.2[a,b]	701.12±10.9[a,b]
PCV (%)	46.61±1.1	39.82±1.2[a]	45.56±1.6[b]	44.71±1.4[a,b]	44.52±1.2[a,b]	45.02±1.5[b]
Hb (g/dl)	16.91±1.2	12.34±0.2[a]	16.72±1.2[b]	16.29±1.4[b]	16.04±1.5[b]	16.60±1.6[b]
MCV (fl)	56.15±0.2	61.47±2.4[a]	55.81±2.5[a,b]	53.98±2.0[b]	54.35±2.2[b]	54.90±2.1[a,b]
MCH(pg)	20.55±1.9	19.07±0.4	19.45±3.9[a,b]	18.71±1.3[a,b]	18.66±1.6[b]	18.99±1.9[a,b]
MCHC (%)	36.66±2.8	31.06±2.1[a]	35.02±2.3[a,b]	34.27±2.[3a,b]	34.11±2.1[a,b]	34.64±2.6[a,b]
Neutrophils (10^3/µl)	3.61±0.5	2.17±0.2[a]	3.52±0.5[b]	2.72±0.3[b]	2.58±0.7[b]	3.22±0.9[b]
Lymphocytes (10^3/µl)	12.92±0.9	9.94±0.2[a]	12.21±0.6[a,b]	10.32±0.3[b]	10.16±0.6[b]	11.51±0.9[b]
Monocytes (10^3/µl)	0.28±0.1	0.16±0.1[a]	0.27±0.3[a,b]	0.25±0.2[b]	0.24±0.2[b]	0.26±0.3[a,b]

Number of experiments (n=6); mean±SD. All the data given in table 1 as mean±**S**D with dissimilar superscript letters (significantly differ at $p < 0.05$). (a) Means significantly differ from control value. (b) Means significantly differ from disease group. There were significant reduction in red blood corpuscles (RBCs), white blood cells (WBCs), Platelets, packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), Neutrophils, Lymphocytes, Monocytes in the disease group as compared to control group after 24 hrs of single dose of azathioprine but there is significant elevation in the value of mean corpuscular volume (MCV). On the other hand when the treatment was given, it shows the protective effect against azathioprine induced reduction in the values of red blood corpuscles, white blood cells, Platelets, packed cell volume, hemoglobin, mean corpuscular hemoglobin concentration, Neutrophils, Lymphocytes, and Monocytes. Mean corpuscular volume value also significantly reduced than disease group.

TABLE 3: HEMATOLOGICAL FINDINGS AFTER 2 WEEKS POST TREATMENT

Test Name	Control Group	Disease Group (Azathioprine)	Standard Group *(Silymarin)*	Test Drug 1 (*Daucus carota L*)	Test Drug 2 (*Camellia sinensis*)	Combination (*Daucus carota L+ Camellia sinensis*)
RBCs (10^6/µl)	8.53±0.2	7.27±0.5[a]	8.61±0.7[b]	7.49±0.6[b]	7.41±1.2[b]	7.99±0.4[b]
WBCs (10^3/µl)	14.85±0.9	13.62±1.4[a]	14.71±0.8[b]	13.98±0.5[b]	13.91±1.2[b]	14.02±0.4[b]
Platelets (10^3/µl)	731.00±28.5	623.33±29.1[a]	691.32±12.3[a,b]	598.95±12.5[a,b]	601.23±12.1[a,b]	636.31±11.9[a,b]
PCV (%)	46.43±1.6	47.41±1.8[a]	46.29±1.8[b]	45.51±1.8[a,b]	45.21±1.5[b]	46.01±1.6[a,b]
Hb (g/dl)	16.52±1.4	16.25±0.5[a]	16.43±1.4[b]	16.11±1.6[b]	16.19±1.6[b]	16.29±1.6[b]
MCV (fl)	54.51±2.5	65.87±2.4[a]	55.12±2.2[b]	56.42±2.5[a,b]	56.99±2.5[a,b]	56.26±2.3[a,b]
MCH(pg)	19.36±0.1	22.58±2.5[a]	19.92±1.7[a,b]	22.14±1.9[a,b]	22.32±1.4[a,b]	21.39±1.5[a,b]
MCHC (%)	35.61±2.5	34.36±2.6	35.52±2.0[a,b]	34.81±2.9[a,b]	34.78±3.1[a,b]	35.09±2.9[b]
Neutrophils (10^3/µl)	3.81±0.4	2.72±0.5	3.62±0.7[b]	2.98±1.3[a,b]	2.81±0.8[b]	3.09±0.4[b]
Lymphocytes (10^3/µl)	10.75±0.9	10.49±1.3	10.69±0.3[a,b]	10.01±0.5[a,b]	10.13±0.2[a,b]	10.32±3.4[a,b]
Monocytes (10^3/µl)	0.32±0.4	0.28±1.5[a]	0.31±0.6[a,b]	0.30±0.7[b]	0.23±0.7[b]	0.31±0.3[b]

Number of experiments (n=6); mean±SD. All the data given in table 2 as means ± SD with dissimilar superscript letters (significantly differ at $p < 0.05$). (a) Means significantly differ from control value. (b) Means significantly differ from disease group. After 2 weeks of post treatment there were significant reduction was seen in red blood corpuscles (RBCs), white blood cells (WBCs), Platelets, Neutrophils, Monocytes in the disease group after 24hrs of single dose of azathioprine as compared to control group but there is significant elevation in the value of mean corpuscular volume (MCVs) and mean corpuscular hemoglobin (MCH) while packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC). Lymphocytes were comparable with control group. On the other hand when the treatment was given it shows the protective effect and returned to normal values. But there is significant reduction in neutrophils and elevation in lymphocytes as compared to normal control value.

TABLE 4: BIOCHEMICAL FINDINGS AFTER 24HRS POST TREATMENT

Test Name	Control Group	Disease Group (Azathioprine)	Standard Group *(Silymarin)*	Test Drug 1 *(Daucus carota L)*	Test Drug 2 *(Camellia sinensis)*	Combination *(Daucus carota L+ Camellia sinensis)*
ALT (U/ml)	27.25±2.2	49.40±3.5[a]	32.28±3.1[ab]	41.32±3.4[a,b]	39.29±2.6[a,b]	35.28±3.[8a,b]
AST (U/ml)	45.00±2.3	151.40±3.6[a]	61.12±2.7[ab]	72.21±2.9[a,b]	73.20±3.1[a,b]	65.26±3.1[a,b]
ALP (IU/I)	110.91±1.3	242.00±1.9[a]	125.01±1.6[a,b]	137.12±2.1[a,,b]	135.21±2.0[a,b]	128.13±2.1[a,b]

All the data given in table 4 as means ± S.D with dissimilar superscript letters (significantly differ at $p < 0.05$) (a) means significantly differ from control value, (b) Mean significantly differ from azathioprine group. After 24 hrs of post treatment of disease group (azathioprine alone) the level of serum hepatic enzymes alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) significantly increased as compared to the control values. On the other hand, when the combination was given the value of alanine transaminase, aspartate aminotransferase, and alkaline phosphatase significantly decreased as compared to disease group but the values of aspartate aminotransferase, alkaline phosphatase, and remains significantly increased than control value.

TABLE 5: BIOCHEMICAL FINDINGS AFTER 2 WEEKS POST TREATMENT

Test Name	Control Group	Disease Group (Azathioprine)	Standard Group *(Silymarin)*	Test Drug 1 *(Daucus carota L)*	Test Drug 2 *(Camellia sinensis)*	Combination *(Daucus carota L+ camellia sinensis)*
ALT (U/ml)	28.36±0.8	37.61±2.6[a]	32.69±3.5[a,b]	42.17±3.2[a,b]	43.34±2.1[a,b]	34.32±3.[6a,b]
AST (U/ml)	50.00±1.5	128.00±3.5[a]	66.24±3.1[a,b]	70.69±3.6[a,b]	70.69±3.9[a,b]	69.03±3.8[a,b]
ALP (IU/I)	123.69±2.7	231.39±2.5[a]	128.12±2.1[a,b]	143.01±2.5[a,b]	143.01±2.1[a,b]	134.36±2.3[a,b]

All the data given in table 5 as means ± S.D with dissimilar superscript letters (significantly differ at $p < 0.05$) (a) means significantly differ from control value (b) means significantly differ from disease group i.e azathioprine alone. After 2 weeks of post treatment of disease group (azathioprine alone), the level of serum hepatic enzymes alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) significantly increased as compared to the control values. Whereas, when the treatment was given to the combination group significantly restores the value of alanine transaminase and alkaline phosphatase as compared to disease group except aspartate aminotransferase whose value was significantly increased than control value.

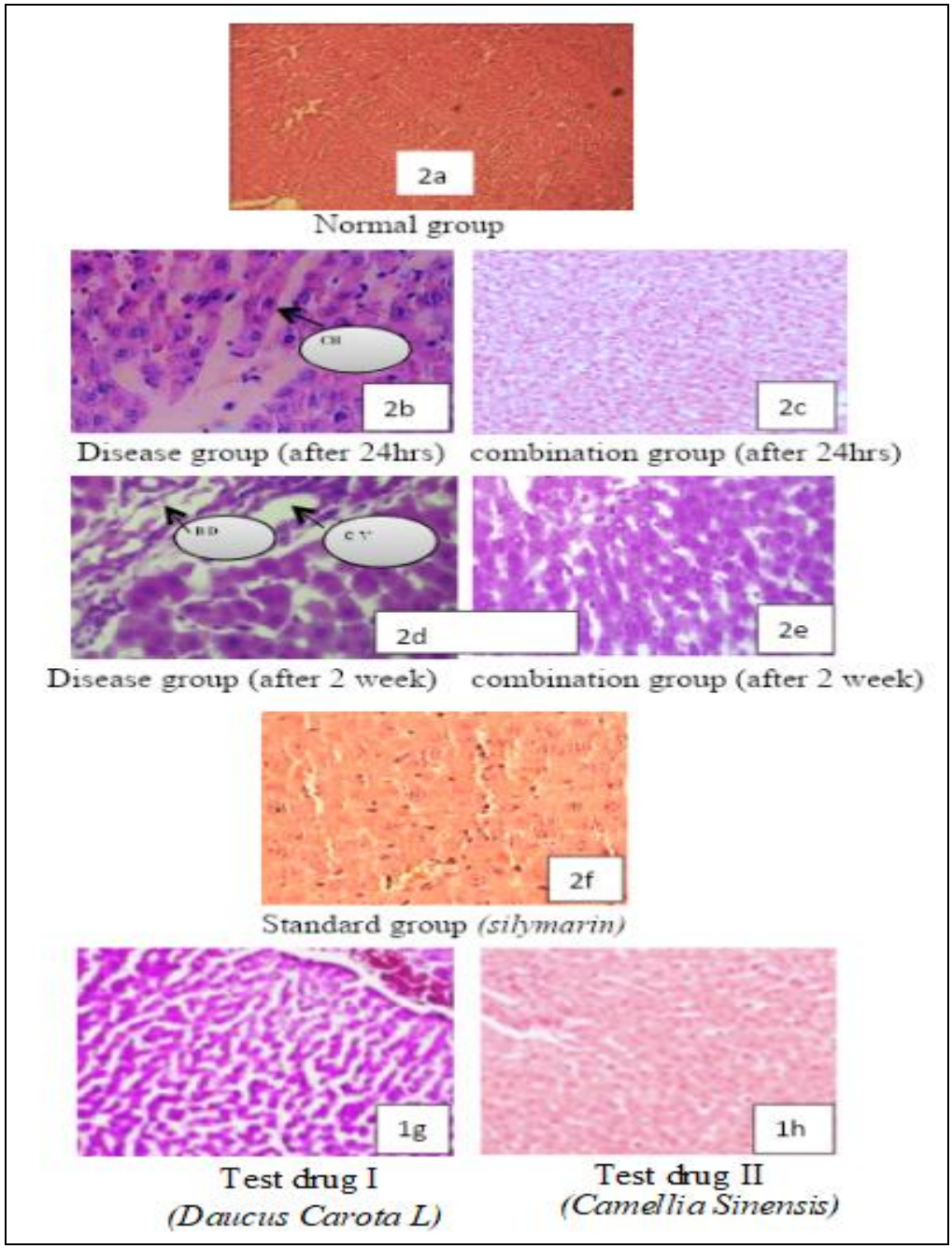


FIG. 2: LIVER SECTION OF RAT, THE CONTROL GROUP SHOWING THE NORMAL HISTOPATHOLOGY OF LIVER I.E NORMAL STRUCTURE OF CENTRAL VEIN (CV) WHICH IS SURROUNDED BY HEPATOCYTES HAVING NO ALTERATION IN (FIG. 2A). LIVER SECTION OF RAT AFTER 24 HRS DISEASE GROUP (AZATHIOPRINE ALONE) SHOWING SOME INFLAMMATORY CELL INFILTRATION IN THE PORTAL AREA AND DIFFUSION IN KUPFFER CELL PROLIFERATION IS ALSO DETECTED IN CYTOMEGALIC HEPATOCYTES (CH) IN (FIG. 2B). ON THE OTHER HAND, LIVER SECTION OF RAT AFTER 24 HRS TREATMENT GROUP (COMBINATION) SHOWING NORMAL HISTOPATHOLOGY IN (FIG. 2C). AFTER 2 WEEKS LIVER SECTION OF RAT (DISEASE GROUP) SHOWING INFLAMMATORY CELL INFILTRATION IN THE PORTAL AREA WITH DILATION IN BILE DUCT (BD), CYTOMEGALIC AND FATTY CHANGES IN (FIG. 2D) AND COMBINATION GROUP SHOWS NORMAL HISTOPATHOLOGY IN (FIG. 2E). THE STANDARD GROUP *(SILYMARIN)* SHOWS NORMAL HISTOPATHOLOGY AFTER 2 WEEKS IN (FIG. 2F). THERE IS LITTLE ALTERATION AND SWELLING BUT A NORMAL APPEARANCE OF HEPATOCYTES IN (FIG. 2G AND 2H) I.E TEST I AND II GROUPS.

DISCUSSION: Hepatotoxicity is a disorder whereby drugs including pharmacological therapies, traditional medicines, herbal and dietary supplements cause liver injury which may lead to the liver fibrosis, cirrhosis and ultimately liver failure if there is long existence of hepatotoxicity.

So it is important to prevent and treat the liver injury at right time to avoid the serious condition [5]. The present work is about to evaluate the Hepatoprotective activity of *Daucus ccarota L* and *Camellia sinensis* extract combination against azathioprine-induced liver injury in rats.

Azathioprine is used as a model in this study.Azathioprine is the oldest pharmacologic immunosuppressive agent in use today. It is the best model to induce liver injury in rats. It suppresses the immune system. Azathioprine 50mg/kg (w/w) i.p was given to group II (disease group) on 8 days as a single day, as a result induction of liver injury within 12 days. Azathioprine converted into its active prodrug 6-mercaptopurine with the help of enzyme glutathione-S-transferase. This reaction consumes the glutathione mother of all antioxidant which is abundantly present in hepatocytes. Azathioprine is also directly metabolized to form inactive metabolites with the help of enzyme xanthine oxidase. Thus, oxidation by xanthine oxidase has the potential to generate reactive oxygen species, as a result lipid peroxidation, directly inhibit proteins synthesis and nucleic acid, mitochondrial depletion. Mitochondrial toxins deplete the ATP level leads to mitochondrial permeability transition. Mitochondria also play important role in cell death by apoptosis which follow the exposure to hepatotoxins and mitochondrial transition either by apoptosis or necrosis [6].

In this study the hepatotoxicity was seen after 24 hrs and 2 weeks post treatment. After 24 hrs of single dose of azathioprine, there were significant reduction in erythrocytes, white blood cells, Platelets, packed cell volume, hemoglobin, mean corpuscular hemoglobin concentration, Neutrophils, Lymphocytes, Monocytes in the disease group after 24 hrs of single dose of azathioprine i.p route as compared to control group but there is significant elevation in the value of mean corpuscular volume. On the other hand, when the treatment was given it shows the protective effect against azathioprine induced reduction in the values of erythrocytes, white blood cells, Platelets, packed cell volume, hemoglobin, mean corpuscular hemoglobin concentration, Neutrophils, Lymphocytes, and Monocytes. Mean corpuscular volume also significantly reduced than azathioprine treated group. After 2 weeks of post treatment there were significant reduction was seen in erythrocytes, white blood cells, Platelets, Neutrophils, and Monocytes in the disease group after 24 hrs of single dose of azathioprine as compared to control group but there was significant elevation in the value of mean corpuscular volume and mean corpuscular hemoglobin while packed cell volume, hemoglobin, mean corpuscular hemoglobin concentration, Lymphocytes was comparable with control group. On the other hand, when the treatment was given, it showed the protective effect and returned to normal values. But there were a significant reduction in neutrophils and elevation in lymphocytes as compared to normal control value.

After 24 hrs of post treatment of azathioprine alone group the level of serum hepatic enzymes alanine transaminase, aspartate aminotransferase, alkaline phosphatase significantly increased as compared to the control values. On the other hand, when the treatment was given to the group of rats the combination significantly decreased the value of alanine transaminase, aspartate aminotransferase, alkaline phosphatase as compared to disease group but the values of aspartate aminotransferase, alkaline phosphatase remains significantly increased than control value. After 2 weeks of post treatment of disease group, the level of serum hepatic enzymes alanine transaminase, aspartate aminotransferase, alkaline phosphatase significantly increased as compared to the control values. On the other hand, when the treatment was given to the group the combination significantly restores the value of alanine transaminase, alkaline phosphatase, as compared to disease group except aspartate aminotransferase whose value was remains significantly increased than control value. The elevation in serum enzymes alanine transaminase, aspartate aminotransferase, alkaline phosphatase is due to the leakage in blood from cytosol of liver.

The rats of the control group showed the normal histopathology of liver after 24 hrs i.e. normal structure of central vein which is surrounded by hepatocytes having no alteration in **Fig. 1A**. After 24 hrs the disease group shows some inflammatory cell infiltration in the portal area and diffusion in Kupffer cell proliferation is also detected in cytomegalic hepatocytes in **Fig. 2B** and treatment

group showed normal histopathology in **Fig. 2C**. After 2 weeks disease group showed inflammatory cell infiltration in the portal area with dilation in bile duct, cytomegalic and fatty changes were also detected in **Fig. 1D** and treatment group shows normal histopathology in **Fig. 2E**. The standard group showed normal histopathology after 2 weeks in **Fig. 2F** on the other hand, little alteration and swelling but normal appearance of hepatocytes in **Fig. 2G** and **2H**.

It is a multistep process that includes some later inflammatory system activation. During Phase I drug metabolism with the help of byproducts polymorphic cytochrome P450 are formed. These hazardous substances might have the potential to initiate the process of liver injury by conjugation during phase II metabolism [7]. Drug induced liver injury is then propagated through subsequent: cell stress, mitochondrial inhibition, and specific immune reactions. Direct cell stress can occur when metabolites attach to certain enzymes, lipids, nucleic acids, or other structures, or when glutathione levels drop. Due to the inhibition of the mitochondrial respiratory chain cause depletion of ATP and build the reactive oxygen species and direct mitochondrial inhibition takes place.

When a medication or its metabolites attach to HLA proteins, which are further recognized as antigens, certain immune responses may be triggered. Neo-antigens are applied to these antigen-presenting cells, causing the antibodies to this cell structure to be activated. These auto-antibodies and reactive metabolites are insufficient to trigger an immunological response. Only when a second signal known as the Danger signal is present do additional reactions take place. These signals, which are caused by oxidative stress or immune-mediated liver injury, activate the signaling pathways. The signals may come from a different drug or host components like bacterial or viral infection. A mild inflammatory reaction can also be the danger signals. Cell stress, direct mitochondrial inhibition, immunological responses, and mitochondrial permeability transition are the final three phases As the permeability increases ATP synthesis gets disturb, which lead to expansion of mitochondrial matrix, also increased permeability of mitochondrial outer membrane. Cytochrome c and apoptotic proteins are consequently released into the cell cytoplasm. Cell stress and immune reaction causes injury in one of the two ways: Cell stress starts the direct pathway that leads to the activation of pro-apoptotic proteins and the inhibition of anti-pro-apoptotic proteins, which causes membrane permeability transition to become active. When an immune response occurs, the extrinsic route will be activated, causing Kupffer cells to release TNF and FAS ligand in response to antigen presentation (FasL).

These TNF and FAS ligands connect to intracellular death receptor and death domain proteins, activate caspase 8, and create a complex that causes death. In addition, Caspase 8 activates the pro-apoptotic Bcl2 proteins, which when combined death receptors result in membrane permeability transition. In the final step when ATP is present, cytochrome C binds to cytoplasmic scaffold protein and pro-caspase 9 to create an Apoptosome, which activates the caspase and causes cytoplasmic and nuclear condensation and fragmentation. Apoptosis is an ATP-dependent mechanism that only happens when membrane permeability transition does not occur quickly. Phagocytosis is used to remove debris.

Apoptosis doesn't result in a breakdown of plasma membrane integrity, which significantly reduces inflammation and the risk of secondary damage.Necrosis develops as a result of membrane permeability transition and ATP depletion impairing mitochondrial activity, causing severe disruption of cell processes which leads to the creation of bleb, actin oxidation, microfilament breakage, cellular swelling, and finally rupture of the plasma membrane. The lysis process results in the production of cytokines, which harms the nearby hepatocytes [8]. *Daucus carota L* and *Camellia sinensis* extract combination used in this study is a natural and excellent source of antioxidants. They balance the level of reactive oxygen species i.e highly reactive species. Oxygen is an extraordinarily reactive atom and capable of becoming part of probably destructive molecules normally known as “free radicals.” free radicals are able to attacking the healthy cells of the body, causing them to lose their shape and function [9].Antioxidants are our first line of defence mechanism towards free radical damage, for retaining health and well-being.

Antioxidants can scavenge loose radicals and defend the human body from oxidative strain. Green tea is rich in polyphenols particularly catechins and gallic acid, but it also contains carotenoids, tocopherols, ascorbic acid (vitamin C), minerals such as Cr, Mn, Se or Zn, and certain phytochemical compounds [10]. These compounds could increase its antioxidant potential. Green tea polyphenols scavenge the reactive oxygen; nitrogen species and chelating redox active transition metal ions; also chelate metal ions like iron and copper to prevent their participation in Fenton and Haber-Weiss reactions [11].

They may also function indirectly as antioxidants by inhibition of the redox sensitive transcription factors, inhibition of 'pro-oxidant' enzymes such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase and induction of antioxidant enzymes such as glutathione-S-transferases and superoxide dismutases [11]. Beta carotene is an efficient quencher of singlet oxygen and comprises several isomers like all-trans and 9-cis β-carotene. They are effective deactivators of electronically excited molecules. They have the ability to protect the cellular membranes and lipoproteins against oxidative damage. They have cooperative effect with other antioxidants like vitamin c generates vitamin E, β-carotene tocopherol from tocopheroxy radical. Vitamin C, E and β-carotene exhibit synergistic effect against reactive oxygen species and reactive nitrogen species [12].

CONCLUSION: Findings clearly indicate that the *Daucus carota L* and *Camellia sinensis* exhibit the hepatoprotective activity by means of their antioxidant, anti-inflammatory and antiapoptotic mechanisms. People are less aware of hepatotoxicity. In most cases of drug induced liver injury, liver can repair itself after withdrawal of suspected drug, but it is not possible in all the cases where the drug induced liver injury is more severe. So, in that case liver transplant is the only option for patients. It is important to be aware of and remove the suspected agent as soon as possible to prevent the progression of chronic liver disease or complete liver failure. There are no specific risk factors for drug induced liver injury, but existing liver disease and genetic factors may be a priority for some people. Treatment of liver damage caused by drugs and herbs includes immediate drug withdrawal and supportive care aimed at alleviating unwanted symptoms. Results clearly showed some harmful effect of azathioprine on haematological, biochemical and histophathological findings. In the present study, we aimed to investigate the synergistic effect to hepatoprotective activity of *Daucus carota L* and *Camellia sinensis* combination against azathioprine induced liver injury in rats. Azathioprine induced oxidative stress; decreased the level of glutathione; elevate liver enzymes alanine transaminase, aspartate aminotransferase, alkaline phosphatase, which leads to lipid peroxidation, destruction of hepatocytes, apoptosis, histopathological changes and hepatic injury. The combination showed the synergistic effect, hepatoprotective activity against highly reactive oxygen species, moreover, maintain the level of glutathione and provide protection against hepatic injury. In this way we provide effective, better treatment for complex ailment pathophysiology of diseases. But we need to explore more in this area of drugs.

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