

## The Potency of Some Natural Products on Dimethyl Benz(A)anthracene(DMBA) Induced Hepatotoxicity in Rats

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### Absrtact

**Aim of the work:**the present study was carried out to investigate the possible ameliorating effects of three herbs: hops(H) 1.5g/kg diet , rosemary(R) 5g/kg diet and cat's claw (C C) 1.7g/kg diet on hepatic toxicity induced by 7,12-Dimethyl benz(A)anthracene , a hydrocarbon involves various negative consequence for human health and ecosystems conversation .**Results:**in this work,48 female rats at 50 day of age were divided into 8 groups; control ,hops(H), rosemary(R), cat's claw(CC),DMBA, DMBA+H,DMBA+R and DMBA+CC groups.Results:the results indicated that a single intraperitoneal (i.p) dose of DMBA (30mg/Kg b.W) caused significant decrease in the percentage of body weight gain, but an increase in the hepatosomatic index. In addition , the results illustrated an increase in the liver malondialdehyde (MDA) contents and hydrogen peroxide levels (H<sub>2</sub>O<sub>2</sub>) accompanied by significant decrease in reduced glutathione (GSH) content and superoxide dismutase (SOD) and glutathione-S-transferase (GST) activities.The results, also reported significant decrease in serum total proteins, total albumin , globulin and liver total protein but serum total bilirubin was significantly elevated in the DMBA intoxicated group. Furthermore, aspartate aminotransaminase (ASAT), alanine aminotransaminase (ALAT) ,  $\gamma$ -glutamyltransferase (GGT) and alkaline phosphatase (ALP) activities were significantly increased in serum but significantly decreased in the liver.

On the other hand, intake of hops, rosemary and cat's claw minimize the disturbances observed in most of the tested parameter's resulted from DMBA administration and improve the liver functions mostly in the following order, rosemary > hops > cat's claw. **Conclusion:** it can be concluded that intake of such herbs (hops , rosemary, cat's claw)may be effective in reducing DMBA toxicity.

**Key words:** natural products, hepatotoxicity,rats.

### Introduction:

7,12-Dimethylbenz[a]anthracene (DMBA) has been recorded to be one of the most widely used polycyclic aromatic hydrocarbons (PAHs), since it is recognized as one of the most potent carcinogens, when compared with other members of this class of chemicals<sup>(1)</sup>.DMBA is well known as cytotoxic, carcinogenic, mutagenic and immunosuppressive agent<sup>(2)</sup>, it supressed both humoral and cell-mediated immune responses<sup>(3)</sup>.Acute treatment of mice by DMBA resulted in a profound hypocellularity in the marrow<sup>(4)</sup>.DMBA is a potent carcinogen which is selectively active in sites such as kidney and liver, and has been widely used as a prototype carcinogen in experimental animal models. Exposure to DMBA induces pathological changes through toxicity occurred to liver and kidney characterized by induction of parenchymal hepatocellular damages including hepatic lesions tumors and cancer risks<sup>(5)</sup>.

Recently, much attention has focused on the role of natural food additives or spices in various disorders reflecting alteration in

cellular function and structure. There is now a growing body of evidence to support the concept that spices, such as Humulus lupulus hops (H) have medicinal properties and may not only alleviate symptoms, but also help to prevent diseases, which belongs to the hemp family ,which has grown wild since ancient times in Europe, Asia, and North America<sup>(6)</sup>.It used as chemopreventive, antiproliferative, an antibiotic , antifungal agent and cytotoxic effects in human cancer cell lines<sup>(7)</sup>. It also promoting healthy kidney functions, treating urinary tract infections , reducing painful urination and tone up liver<sup>(8)</sup>.Alternatively, rosemary (R) belongs to lamiaceae family (labiateae) is a woody , perennial herb with fragrant, evergreen, needle-like leaves<sup>(9)</sup> . It is a flowering plant that grows in Mediterranean countries, southern Europe and in the littoral region through Minor Asia areas wildly. It has been proposed that rosemary and its constituents have a therapeutic potential in treatment or prevention of inflammatory diseases, hepatotoxicity, renal toxicity and heart diseases <sup>(10)</sup>.Additionally, cat's claw (CC)

is a woody vine found in the tropical jungles of south and central America, which derives its name from its claw-shaped thorns. It is used as an alternative medicine in the treatment of a variety of ailments. It is a supplement in the treatment of many human disorders including inflammations, cancer and infections<sup>(11)</sup>. It also used to kill cancer cells, support the immune system<sup>(12)</sup>, treat cirrhosis<sup>(13)</sup> and kidney problems<sup>(14)</sup>.

Based on these information, the present study was designed to investigate the possible protective effects of the three mentioned herbs against hepatic toxicity resulted from DMBA hydrocarbon exposure.

## MATERIAL AND METHODS

### 1-Experimental animals:

This study was performed on forty eight female wistar albino rats (*Rattus norvegicus*), 50 day of age, initially weighing (50±10g). They were obtained from Helwan Animal Farm, Cairo, Egypt. They were housed in stainless steel cages at a well-ventilated animal house. Rats were permitted adequate standard diet and given water ad libitum for two weeks of acclimatization prior to the experimentation. The animals were weighed at the beginning of the experiment and weekly till the end of the experiment (4 months).

### 2- Experimental groups and mood of treatment:

The experimental animals were randomly divided into eight groups, each of six rats as follows:

- **Control group (C):** received normal laboratory diet without any supplementation.
- **Hops treated group (H):** received normal laboratory diet supplemented with grinding hops leaves and flower powder in a dose of 1.5g/kg diet<sup>(15)</sup>.
- **Rosemary treated group (R):** received normal laboratory diet supplemented with grinding rosemary leaves powder in a dose of 5g/kg diet<sup>(16)</sup>.
- **Cat's claw group (CC):** received normal laboratory diet supplemented with grinding cat's claw powder in a dose of 1.7g/kg diet<sup>(17)</sup>.
- **DMBA intoxicated group (DMBA):** received a single i.p dose of DMBA (30 mg/kg b.w.)<sup>(18)</sup>.
- **DMBA + hops group**

**(DMBA+H):** received dietary supplement of hops powder (1.5g/kg diet) and a single i.p dose of DMBA (30 mg/kg b.w. at 50 day of age).

- **DMBA + rosemary group (DMBA+R):** received dietary supplement of roemary powder (5g/kg diet) and a single i.p dose of DMBA (30 mg/kg b.W. at 50 day of age).
- **DMBA + cats claw group (DMBA+CC):** received dietary supplements of cat claw powder (1.7 g/kg diet) and a single i.p dose of DMBA (30 mg/kg b.w. at 50 day of age).

### 3- Blood and tissue sampling:

At the end of the experimental period, rats were weighed and kept individually in metabolic cages for 24 hours for urine collection, that were centrifuged then clean urine samples were stored at -20 °C until analysis. Animals fasted overnight then sacrificed by cervical dislocation and blood samples were collected in non - heparinized tubes, then were centrifuged at 860XG for 15 min. at 4°C for getting sera which were frozen at - 20 °C for future biochemical analysis.

Thereafter, the liver was removed, cleaned and weighed. Small, weighed specimens were then homogenized in known volume of distilled water, using homogenizer surrounded with ice jacket and the homogenates were kept frozen at - 20 °C until being analyzed.

The end product of lipid peroxidation **malondialdehyde (MDA)** was determined as thiobarbituric acid reactive substance (TBARS)<sup>(19)</sup> while **hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) concentration** was estimated in serum and liver homogenates, using kit from biodiagnostic, Giza, Egypt kit<sup>(20)</sup>.

**Antioxidant biomarkers, GSH content, SOD and GST activities** were measured<sup>(20,21,22)</sup>.

**Total protein and albumin** were determined using kit from Biodiagnostic, Giza, Egypt<sup>(23,24)</sup> while **total bilirubin concentration** was estimated<sup>(25)</sup>.

However, **Transaminases (ASAT and ALAT) and GGT enzyme activities**, were estimated<sup>(26,27)</sup>.

### Statistical analysis:

All data are represented as means±SE. One way analysis of variance (One-way ANOVA) followed by Least Significant Difference (LSD) test was used to determine differences among means of investigated

groups. The differences were considered to be statistically significant at  $P < 0.05^{(28)}$ .

### Results

The obtained data in the present study are presented in Table 1 ,2,3 and 4.

Table (1) showed that a single i.p dose of DMBA (30mg/Kg b.W) caused alterations in comparison with the control group including significant decrements in the percentage of body weight (bw) gain , GSH contents, SOD and GST activities and this was accompanied by significant increase in hepato somatic index,MDA contents and  $H_2O_2$  levels Meanwhile, the intake of hops, rosemary and cat's claw herbs ameliorated the above mentioned parameters.

As shown in Table(2)DMBA treated rats showed significant decreases in serum total protein, albumin and globulin contents and liver total protein contents in contrast to significant increase in serum total bilirubin when compared with the control group, however, supplementation of DMBA treated rats with hops(H), rosemary(R)and cat's claw (CC)herbs significantly improved the

reduction of serum and hepatic total protein contents as well as serum albumin and globulin contents and significantly decreased serum total bilirubin in comparison with DMBA group, but such values are still significantly differ from the control levels. It was noted that R was the best in ameliorating serum albumin, globulins and total bilirubin.

Table (3) represent serum and liver ASAT, ALAT, GGT and ALP activities in different animal groups, significant increase in serum ASAT, ALAT,GGT and ALP activities with significant decrease in their activities in liver of DMBA treated rats were recorded when compared to control group. On the other hand, the administration of hops, rosemary and cat's claw herbs with DMBA improved both serum and liver enzymatic activity by causing reduction of serum ASAT, ALAT ,GGT and ALP activities concomitant to an enhancement of their hepatic activities when compared with DMBA group. Such improvement was slight and still, significantly different from the control values to great extent.

**Table (1): Percentage of body weight gain , Relative liver weight, lipid peroxidation product(MDA) , hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) levels ,reduced glutathione (GSH) contents, superoxide dismutase (SOD) and glutathione-S-transferase (GST) activities in different animal groups.**

Group		Control	H (1.5g/k g food)	R (5g/ Kg food)	C C (1.7g/ Kg food)	DMBA (30mg/ Kg bw)	DMBA + H	DMBA + R	DMBA + C C
Tissue									
%of Body weight gain	Mean	364.4	362.4	370.4	373.0	257.5	302.5	320.0	310.0
	SE	± 8.0	± 6.2	± 7.0	± 7.2	± 8.9 <sup>a</sup>	± 8.2 <sup>a,b</sup>	± 10.2 <sup>a,b</sup>	± 9.0 <sup>a,b</sup>
Hepatosomatic index	Mean	3.48	3.55	3.52	3.60	5.60	4.61	4.27	4.53
	SE	± 0.12	± 0.08	± 0.12	± 0.14	± 0.12 <sup>a</sup>	± 0.80 <sup>a,b</sup>	± 0.16 <sup>a,b</sup>	± 0.12 <sup>a,b</sup>
	*		+2.0	+1.2	+3.7	+60.9	+32.5	+22.7	+30.2
	**						-17.7	-23.78	-19.10
MDA (nmol/g)	Liver	63.90	61.70	63.50	60.00	95.50	73.10	71.10	74.40
		± 1.6	± 1.6	± 2.0	± 1.2	± 2.5 <sup>a</sup>	± 2.0 <sup>a,b</sup>	± 1.2 <sup>a,b</sup>	± 1.6 <sup>a,b</sup>
	*		-3.5	-0.7	-6.1	+49.5	+14.4	+11.2	+16.4
	**						-23.4	-29.6	-22.1
H <sub>2</sub> O <sub>2</sub> (mM/g)	Liver	0.295	0.303	0.250	0.284	0.450	0.400	0.360	0.370
		± 0.008	± 0.004	± 0.008	± 0.012	± 0.008 <sup>a</sup>	± 0.012 <sup>a,b</sup>	± 0.14 <sup>a,b</sup>	± 0.008 <sup>a,b</sup>
	*		+2.3	-15.3	-3.7	+52.5	+35.6	+39.0	+25.4
	**						-11.1	-20.0	-17.8
GSH (mg/g)	Liver	4.00	4.09	4.15	4.20	2.54	3.51	3.57	3.68
		± 0.12	± 0.16	± 0.08	± 0.16	± 0.08 <sup>a</sup>	± 0.25 <sup>a,b</sup>	± 0.06 <sup>a,b</sup>	± 0.12 <sup>a,b</sup>
	*		+2.3	+3.8	+5.0	-36.5	-12.3	-10.2	-8.0
	**						+38.1	+40.6	+44.9
SOD (U/g)	Liver	100.20	103.10	103.00	103.80	65.90	89.90	91.10	90.50
		± 2.9	± 3.7	± 4.1	± 4.1	± 2.0 <sup>a</sup>	± 3.0 <sup>a,b</sup>	± 2.9 <sup>a,b</sup>	± 2.0 <sup>a,b</sup>
	*		+2.9	+2.9	+3.6	-34.2	-10.3	-9.1	-9.7
	**						+36.4	+38.1	+37.2
GST(m ol/g)	Liver	0.192	0.197	0.206	0.201	0.136	0.151	0.178	0.153
		± 0.004	± 0.050	± 0.030	± 0.040	± 0.020 <sup>a</sup>	± 0.010 <sup>a,b</sup>	± 0.010 <sup>a,b</sup>	± 0.004 <sup>a,b</sup>
	*		+2.6	+7.3	+4.7	-29.2	-21.4	-7.3	-20.3
	**						+11.0	+30.9	+12.2

- Data are presented as means ±SE (n = 6 for each group).
- **a** and **b** significant changes at p≤0.05 comparing to control and DMBA intoxicated groups respectively.
- \* and \*\*are % of change comparing to control and DMBA intoxicated groups respectively.
- **H:** Hops      **R:** Rosemary      **CC:** Cat's claw      **DMBA:** 7,12-Dimethyl benz(a)anthracene.

**Table (2): Serum and liver total proteins contents, serum total albumin , globulin, A/G ratio and total bilirubin concentration in different animal groups.**

Group		Control	H (1.5g/ Kg food)	R (5g/Kg Food)	C C (1.7g/ Kg food)	DMBA (30mg/ Kg bw)	DMBA + H	DMBA + R	DMBA + CC
Serum	Total protein (mg/dL)	7.90 ± 0.30	8.19 ± 0.22	8.07 ± 0.33	8.06 ± 0.20	4.50 ± 0.08 <sup>a</sup>	5.31 ± 0.20 <sup>a,b</sup>	5.25 ± 0.20 <sup>a,b</sup>	5.33 ± 0.20 <sup>a,b</sup>
		*	<b>+3.70</b>	<b>+2.20</b>	<b>+2.02</b>	<b>-43.04</b>	<b>-32.78</b>	<b>-33.54</b>	<b>-32.53</b>
		**					<b>+17.77</b>	<b>+16.66</b>	<b>+18.44</b>
	Albumin (mg/dL)	5.24 ± 0.17	5.66 ± 0.14	5.48 ± 0.16	5.51 ± 0.20	2.80 ± 0.08 <sup>a</sup>	3.21 ± 0.12 <sup>a,b</sup>	3.24 ± 0.08 <sup>a,b</sup>	3.12 ± 0.11 <sup>a,b</sup>
		*	<b>+8.0</b>	<b>+4.6</b>	<b>+5.2</b>	<b>-46.4</b>	<b>-38.7</b>	<b>-38.2</b>	<b>-40.5</b>
		**					<b>+14.6</b>	<b>+15.7</b>	<b>+11.4</b>
	Globulin (mg/dL)	2.74 ± 0.08	2.53 ± 0.04	2.59 ± 0.08	2.55 ± 0.04	1.70 ± 0.04 <sup>a</sup>	2.10 ± 0.06 <sup>a,b</sup>	2.01 ± 0.04 <sup>a,b</sup>	2.21 ± 0.05 <sup>a,b</sup>
		*	<b>-7.6</b>	<b>-5.5</b>	<b>-6.9</b>	<b>-38.0</b>	<b>-23.4</b>	<b>+26.6</b>	<b>-19.3</b>
		**					<b>+30.0</b>	<b>+62.3</b>	<b>+30.0</b>
Total bilirubin (mg/dL)	1.803 ± 0.04	1.450 ± 0.10	1.393 ± 0.04	1.327 ± 0.04	2.886 ± 0.10 <sup>a</sup>	2.200 ± 0.08 <sup>a,b</sup>	2.137 ± 0.07 <sup>a,b</sup>	2.310 ± 0.08 <sup>a,b</sup>	
	*	<b>-19.4</b>	<b>-22.7</b>	<b>-26.4</b>	<b>+60.1</b>	<b>+22.2</b>	<b>+18.8</b>	<b>+28.1</b>	
	**					<b>-23.60</b>	<b>-25.80</b>	<b>-20.0</b>	
Liver	Total Protein (mg/g)	2.00 ± 0.08	2.10 ± 0.04	2.25 ± 0.08	2.13 ± 0.04	1.02 ± 0.04 <sup>a</sup>	1.39 ± 0.05 <sup>a,b</sup>	1.37 ± 0.04 <sup>a,b</sup>	1.40 ± 0.04 <sup>a,b</sup>
		*	<b>+5.0</b>	<b>+12.5</b>	<b>+6.5</b>	<b>-49.0</b>	<b>-30.0</b>	<b>-31.5</b>	<b>-30.2</b>
		**				<b>+36.0</b>	<b>+34.1</b>	<b>+36.6</b>	

- Data are presented as means ±SE (n = 6 for each group).
- **a** and **b** significant changes at p≤0.05 comparing to control and DMBA intoxicated groups respectively.
- \* and \*\* are % of change comparing to control and DMBA intoxicated groups respectively.
- **H**: Hops      **R**: Rosemary      **CC**: Cat's claw      **DMBA**: 7,12-Dimethyl benz(a)anthracene.

**Table (3): Serum and liver aspartate aminotransaminase (ASAT), alanine aminotransaminase (ALAT) and glutamyl-S- transferase (GGT) activities in different animal groups:**

Group		Control	H (1.5g/ Kg food)	R (5g/ Kg food)	CC (1.7g/ Kg food)	DMBA (30mg/ Kg bw )	DMBA + H	DMBA + R	DMBA + CC
Parameters									
ASAT	Serum (U/L)	63.90 ± 1.6	63.20 ± 1.7	58.70 ± 1.2	66.10 ± 1.4	117.50 ± 2.9 <sup>a</sup>	108.40 ± 2.0 <sup>a,b</sup>	85.20 ± 1.0 <sup>a,b</sup>	112.10 ± 1.6 <sup>a</sup>
	*		<b>-1.2</b>	<b>-8.3</b>	<b>+3.3</b>	<b>+83.7</b>	<b>+69.4</b>	<b>+33.2</b>	<b>+75.2</b>
	**						<b>-7.8</b>	<b>-27.5</b>	<b>-4.6</b>
	Liver (U/g)	34.20 ± 0.8	35.00 ± 0.4	35.30 ± 1.6	35.20 ± 1.2	26.10 ± 0.4 <sup>a</sup>	30.10 ± 0.8 <sup>a,b</sup>	30.50 ± 0.4 <sup>a,b</sup>	31.20 ± 0.4 <sup>a,b</sup>
	*		<b>+2.4</b>	<b>+3.2</b>	<b>+3.1</b>	<b>-23.8</b>	<b>-11.9</b>	<b>-10.7</b>	<b>-8.8</b>
	**						<b>+15.6</b>	<b>+17.1</b>	<b>+19.6</b>
ALAT	Serum (U/L)	15.30 ± 0.4	13.50 ± 0.3	13.80 ± 0.5	14.50 ± 0.4	43.50 ± 1.2 <sup>a</sup>	34.80 ± 0.8 <sup>a,b</sup>	35.30 ± 0.8 <sup>a,b</sup>	36.60 ± 0.4 <sup>a,b</sup>
	*		<b>-11.8</b>	<b>-10.9</b>	<b>-5.6</b>	<b>+184.1</b>	<b>+127.4</b>	<b>+130.3</b>	<b>139.1</b>
	**						<b>-20</b>	<b>-18.9</b>	<b>-15.9</b>
	Liver (U/g tissue)	31.00 ± 0.8	39.70 ± 0.4	37.50 ± 0.4	36.30 ± 0.8	9.80 ± 0.4 <sup>a</sup>	18.00 ± 0.8 <sup>a,b</sup>	17.00 ± 0.4 <sup>a,b</sup>	15.90 ± 0.4 <sup>a,b</sup>
	*		<b>+28.0</b>	<b>+21.0</b>	<b>+17.1</b>	<b>-68.3</b>	<b>-41.9</b>	<b>-45.2</b>	<b>-48.7</b>
	**						<b>+83.1</b>	<b>+72.9</b>	<b>+61.6</b>
GGT	Serum (U/L)	25.90 ± 2.0	29.30 ± 3.0	36.30 ± 3.2	34.80 ± 2.5	72.40 ± 5.0 <sup>a</sup>	59.60 ± 5.2 <sup>a,b</sup>	65.10 ± 4.9 <sup>a,b</sup>	63.00 ± 4.0 <sup>a,b</sup>
	*		<b>+13.3</b>	<b>+40.4</b>	<b>+34.6</b>	<b>+179.9</b>	<b>+130.6</b>	<b>+151.8</b>	<b>+143.7</b>
	**						<b>-17.6</b>	<b>-10.0</b>	<b>+13.0</b>
	Liver (U/g)	28.65 ± 2.1	31.91 ± 1.5	29.59 ± 1.5	33.11 ± 1.9	14.54 ± 1.2 <sup>a</sup>	24.37 ± 1.4 <sup>a,b</sup>	20.87 ± 2.2 <sup>a,b</sup>	23.29 ± 2.3 <sup>a,b</sup>
	*		<b>+11.3</b>	<b>+3.3</b>	<b>+15.6</b>	<b>-49.3</b>	<b>-15.0</b>	<b>-27.2</b>	<b>-18.7</b>
	**						<b>+67.6</b>	<b>+43.5</b>	<b>+60.2</b>
ALP	Serum (U/L)	65.70 ± 1.6	63.70 ± 1.2	61.10 ± 1.6	62.30 ± 0.8	80.10 ± 2.0 <sup>a</sup>	70.60 ± 1.6 <sup>a,b</sup>	72.30 ± 1.2 <sup>a,b</sup>	72.30 ± 1.6 <sup>a,b</sup>
			<b>-3.0</b>	<b>-7.0</b>	<b>-5.2</b>	<b>22.0+</b>	<b>+7.5</b>	<b>+10.0</b>	<b>+10.1</b>
							<b>-11.9</b>	<b>-9.8</b>	<b>-9.7</b>
	Liver (U/g)	49.20 ± 1.2	55.40 ± 1.2	53.30 ± 1.3	55.20 ± 1.6	31.30 ± 1.0 <sup>a</sup>	40.00 ± 1.2 <sup>a,b</sup>	40.10 ± 1.2 <sup>a,b</sup>	39.0 ± 1.6 <sup>a,b</sup>
			<b>+13.0</b>	<b>+8.4</b>	<b>+12.2</b>	<b>-36.3</b>	<b>-18.8</b>	<b>-18.5</b>	<b>-21.2</b>
							<b>+27.6</b>	<b>+27.9</b>	<b>+23.8</b>

- Data are presented as means ±SE (n = 6 for each group).
- **a** and **b** significant changes at p≤0.05 comparing to control and DMBA intoxicated groups respectively.
- \* and \*\* are % of change comparing to control and DMBA intoxicated groups respectively.
- **H:** Hops      **R:** Rosemary      **CC:** Cat's claw      **DMBA:** 7,12-Dimethyl benz(a)anthracene.

**Discussion:**

The present study revealed that 7,12-dimethyl benz(a)anthracene (DMBA) increased malondialdehyde concentration (MDA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) levels in the liver tissue. These results are similar to the data reported by **Amin** <sup>(29)</sup>, **Mirunalini** <sup>(18)</sup> who indicated that DMBA intake produced oxidative stress in liver of rats. Similarly, a significant increase in liver thiobarbituric acid reactive substances after stimulation by DMBA was reported in rat by **Paliwal** <sup>(5)</sup>. In fact, DMBA is metabolized by the mixed function oxidases system (MFO) often results in the formation of oxyradicals which bind covalently to nucleophilic sites on cellular macromolecules thereby eliciting cancerous responses <sup>(30)</sup>. Generally, Increased incidence of oxidative stress and lipid peroxidation are implicated in carcinogenic process <sup>(31)</sup>. Thereby oxidative stress induced due to the generation of free radicals, and the decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis <sup>(32)</sup>. Prior, emerging evidences suggested that DMBA induced the production of reactive oxygen species (ROS) that resulted in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems <sup>(33)</sup>. Furthermore, It has been postulated that DMBA a potent carcinogen which is selectively active in sites such as kidney and liver mediates carcinogenesis through formation of DNA adducts, DNA damage, generating excess reactive oxygen species and by producing chronic inflammation <sup>(34)</sup>.

Moreover, ROS can diffuse from the site of generation to other targets within the cells or even propagate the injury outside the cells <sup>(35)</sup>. Indeed, Lipid peroxidation (LPO) causes alterations in membrane integrity, therefore, causing impairment of major metabolic functions, which are dependent on membrane structure and integrity <sup>(36)</sup>. The changes in lipid peroxidation production reactions and antioxidant defense systems were associated with changes in a variety of biochemical pathways <sup>(5)</sup>.

The present study exhibited decreased reduced glutathione (GSH) content, superoxide dismutase (SOD) and glutathione-S-transferase (GST) activities in DMBA treated group. These findings agreed with the

antecedent studies of **Mirunalini** <sup>(18)</sup> and **Koul** <sup>(35)</sup>, they recorded that GSH content, SOD and GST activities decreased in liver and kidney tissues of rats intoxicated by DMBA. However, the reduction in the tested antioxidants could be due to its consumption in clearing the free radicals inside the cells and indicated a high degree of free radical production and LPO occurrence. Also, the lowered levels of the mentioned antioxidants in DMBA, is in accordance with the suggestion of **Sharma** <sup>(37)</sup> may be due to altered antioxidant status caused by DMBA induced carcinogenesis events.

The reduced glutathione (GSH) serves as a marker for evaluation of oxidative stress and it acts as an antioxidant at both extra cellular and intracellular levels. GSH and GSH dependent enzymes are involved in scavenging the electrophilic moieties involved in cancer initiation. This confirmed by the early study of **Comporti** <sup>(38)</sup> who observed decreased activity of GSH in cancer-bearing animals.

The noticeable decrease in GSH content observed on DMBA exposed rats, that could be well correlated with the increased levels of lipid peroxidation observed in DMBA treated mice. It is evidently clear that exposure to carcinogens such as polycyclic aromatic hydrocarbon (as DMBA) causes a decrease in the GSH levels in the target organs such as liver and kidney <sup>(35)</sup>.

On other side, the data obtained by the present study illustrated that administration of hops (H), rosemary (R) or cat's claw (CC) herbs to DMBA treated rats caused a significant decrease in the levels of MDA and H<sub>2</sub>O<sub>2</sub> in the liver and kidney but greatly enhanced hepatic GSH contents and SOD and GST enzymes activities when compared with DMBA intoxicated rats. With respect to hops (H), its flavonoids have previously exhibited the ability to scavenge reactive nitrogen and oxygen species by inhibiting neuronal nitric oxide synthase <sup>(39)</sup>. However, hops is used as a free radical scavenging and antitumor properties <sup>(6)</sup>, toning up the liver, assisting a sluggish gall bladder and increasing the flow of urine <sup>(8)</sup>, thus can improving both the liver and kidney functions. In hops, the Prenylated flavonoids, including isoxanthohumol and xanthohumol (XN) have exhibited high chemopreventive, antiproliferative, and cytotoxic effects in human cancer cell lines

lines<sup>(40), (7)</sup>. Its mechanisms of protection have been proposed to be inhibition of metabolic activation, induction of detoxifying enzymes and antioxidant activity. Moreover, xanthohumol (XN) a prenylated chalcone from hops is able to scavenge reactive oxygen species, including hydroxyl- and peroxy radicals, and to inhibit superoxide anion radical and nitric oxide production<sup>(41)</sup>. So, xanthohumol had the highest activity in total oxygen radical absorbance capacity as well as singlet oxygen absorbance capacity<sup>(42)</sup>.

Furthermore, hop bitter acids, a family of bitter compounds derived from the H plant, have been reported to exert a wide range of effects, both in vitro and in vivo. They exhibit potential anticancer activity by inhibiting cell proliferation and angiogenesis, or by inducing apoptosis, and by increasing the expression of cytochrome P450 detoxification enzyme<sup>(43)</sup>. Also, H acids are effective against inflammatory and metabolic disorders.

Concerning, the elevated levels of GSH by rosemary herb in R+DMBA group, it indicated a protected cellular proteins against oxidation through glutathione redox cycle and also showed direct detoxifying of the reactive species<sup>(44)</sup>. These results are in harmony with those reported by **Gutiérrez**<sup>(45)</sup> who illustrated that dietary supplementation with **R** increased the antioxidative capacity and lowered lipid peroxidation by stabilizing the membrane integrity. Such results were confirmed by the recent study showing that inclusion of rosemary powder to Y-irradiated or ethanol ingested rats provided anti-lipoperoxidant activity, as it reduced the formation of MDA and significantly elevated GSH content and the activity of SOD in liver<sup>(46)</sup>. Moreover, **parmer**<sup>(47)</sup> observed significant decreases in oxidative stress markers including serum TBARS and nitric oxide (NO) and an increase in serum enzymatic GST and non-enzymatic antioxidants in rats treated with **R** extract.

Similarly, **Bakirel**<sup>(48)</sup> found that long term administration with high dose of **R** extract had reversed the mentioned activities of the antioxidant enzymes, perhaps, by decreasing the oxidative stress as evidenced by decreased lipid peroxidation. The authors proposed that *Rosmarinus officinalis* extract, due to presence of several bioactive antioxidant principles, such as rosmarinic acid,

carnosol and rosmaridiphenol and their synergistic properties, may cause an improving effect in the antioxidant status.

Regarding cat's claw (CC), it has antitumoral properties and acts as a free radical scavenger and provides reactive oxygen species (ROS) homeostasis.<sup>(49)</sup> and increase GST activity<sup>(50)</sup>. It had immunostimulating and antioxidant properties, probably related to the high concentration of flavonoids that act against ROS, decreasing the oxidative stress in the inflammatory process<sup>(51)</sup>.

It has been exhibited that an aqueous extract of the cat's claw bark negated the cellular toxicity associated with peroxy nitrite, a powerful oxidant formed from the interaction of nitric oxide and superoxide (the radical precursors to a family of potent, reactive species)<sup>(52)</sup>. Alternatively, recorded that an important in vitro free radical-scavenging activity was detected at various concentrations of cat's claw (CC) extract. Treatment by CC also resulted in an increased CAT activity in liver<sup>(50)</sup>.

The present study suggested that DMBA intoxication caused depletions in serum and liver total protein, serum albumin, globulin contents and A/G ratio but increased level of serum total bilirubin. These results are in coordination with the results obtained by **Vijayabaskaran**<sup>(58)</sup> and **Parmar**<sup>(47)</sup> and may be due to hepatic and nephritic inflammation and oxidative damage and / or change in protein synthesis and metabolism as well as increased protein oxidation<sup>(53)</sup> and indicating disturbed liver function. In parallel, DMBA exposure was shown to induce pathological changes through toxicity occurred to liver characterized by induction of parenchymal hepatocellular damage including hepatic lesion tumor and cancer risks<sup>(5)</sup>, thus affecting protein metabolism and disturbing body protein hemostasis. The increased nitric oxide production (NO), that follows DMBA-induced oxidative stress plays a role in liver damage induction. The hepatocellular injury attributed to NO may be due to either its direct cytotoxicity or its reaction with superoxide to produce the toxic nitrogen metabolite peroxy nitrite<sup>(54)</sup>.

On the other hand, the results of the present study indicated that hops (H), rosemary (R) and cat's claw (CC) caused improvement in the mentioned parameters as

evidence by the elevation in serum and liver total protein, serum total albumin, total globulin and A/G ratio in contrast to decreasing the level of serum total bilirubin, perhaps through it may due to scavenging free radicals<sup>(6)</sup>. However, Prenylated flavonoids of H including isoxanthohumol and xanthohumol have exhibited high chemopreventive, antiproliferative, and cytotoxic effects in human cancer cell lines<sup>(7)</sup> by inhibiting metabolic activation, induction of detoxifying enzymes and antioxidant activity<sup>(40)</sup>.

For rosemary (R) it was found that the meal contain R leaves increased total protein, total albumin and total globulin and decreased A/G ratio in serum of chicken<sup>(47)</sup> <sup>(55)</sup>. The increase in globulin fraction indicates the effective role of rosemary in increasing immunity due to its role in protecting developing cells and inhibiting non-enzymatic oxidation<sup>(56)</sup>. Also, R both prevented and reversed CCl<sub>4</sub>-induced damage as evidenced by a reduction in bilirubin levels, suggesting an improvement in biotransformation<sup>(45)</sup>.

Moreover, R has a reactive radical scavenging properties which stabilized the reactive radicals thus converting them into stable species before reaching the parenchyma of the liver<sup>(57)</sup>. Further, it lead to induction of the hepatic endogenous antioxidant enzymes; improvement of hepatic excretory function of bile and the amelioration of erythrocyte integrity against CCl<sub>4</sub> damaging effect which could explain the improved serum bilirubin level and enhance the antioxidant status and function of the liver and kidney.

Cat's claw had antioxidant properties, probably due to the high concentration of flavonoids that act against reactive oxygen species, decrease the oxidative stress in the inflammatory process<sup>(51)</sup>, kill cancer cells, support and stimulate the immune system<sup>(12)</sup>, treat cirrhosis<sup>(13)</sup>.

The present study illustrated that DMBA administration elevated ASAT, ALAT, GGT and ALP enzyme activities in the serum of rats. While, it depleted these enzymes in liver tissues. These results were in accordance with those obtained by Vijayabaskaran<sup>(58)</sup>. The elevated activities of serum GGT observed in DMBA treated group is considered as indicative of DMBA induced hepatic damage<sup>(5)</sup> and subsequent leakage of these enzymes into circulation. Moreover, the

elevation in the level of ASAT, ALAT, ALP in serum of DMBA treated rats may be attributed to cell membrane damage, leading to various enzymes leak down to the circulatory fluid<sup>(58)</sup>. Similarly, Al-Attar<sup>(60)</sup> reported decreasing in ASAT, ALAT activities in hepatocytes of DMBA-intoxicated *Ranaridibunda*, frog, the decreased liver ALAT activity may be correlated with the fact that there is deficient conversion of alanine to pyruvate which enters into Krebs cycle to compensate for energy requirement, alternatively, ASAT is specific for glutamate and ketoglutarate but reacts with nearly all amino acids. The depletion in the activities of ASAT and ALAT enzyme following DMBA exposure indicates disruption of link between carbohydrate and protein metabolism providing source of keto acids for Krebs cycle and gluconeogenesis<sup>(13)</sup>.

In addition, the decrease in tissue ALP in DMBA treated rats is because it is a membrane bound enzyme that present in the bile pole of hepatocytes and also in the pinocytic vesicle and Golgi complex. It is present on all cell membranes where active transport occurs, and hydrolase and transphosphorylase in function. However, the decrease in ALP may be taken as index of hepatic parenchymal damage and hepatocytic necrosis<sup>(5)</sup>. Inhibition of hepatic ALP reflects alterations in protein synthesis and uncoupling of oxidative phosphorylation<sup>(61)</sup>. The decrease in ALP by stressors probably indicates an altered transport of phosphate and an inhibitory effect on the cell growth and proliferation.

In the present work, administration of hops (H), rosemary (R) and cat's claw (CC) ameliorated the enzyme activities by depleting serum ASAT, ALAT, GGT and ALP in rats, meanwhile elevating them in the liver compared to DMBA group. Such amelioration of serum and liver enzyme activities could be attributed to the antioxidant properties of the herbal constituents and their ability to scavenge the free radicals as well as their anti-inflammatory action hence protecting cellular membranes integrity from oxidative damage DMBA toxicity<sup>(39)</sup>, <sup>(48)</sup>, <sup>(50)</sup>. A mice study revealed the chemopreventive activity of H flavonoids<sup>(7)</sup> that appears to be due to induction of quinone reductase (QR) (a hepatic phase II detoxifying enzyme)<sup>(62)</sup> and reduction of the expression of CYP1A1 (a phase I

enzyme that activates chemical carcinogens. QR is primarily a cytosolic flavoprotein that protects cells against the toxicity of xenobiotics by catalyzing the reduction of a wide variety of quinines and quinoneimines<sup>(63)</sup>.

Rosemary crude (R) extract and its constituents carnosol and carnosic acid have been reported to show chemopreventive benefits in in vivo antitumorigenesis studies, with the activation of phase I and phase II detoxifying enzymes being implicated as the mechanisms of action<sup>(64)</sup>. Rosemary leaves were found to contain high antioxidant activity<sup>(65)</sup>. Rosmarinic acid, diterpenoids, carotenoids and alpha-tocopherol<sup>(66),(67)</sup>. It has been documented that as principal antioxidant constituents of R water extract has hepatoprotective, and antitumorigenic activities<sup>68</sup>. Carnosic acid also provides protection from the liver carcinogen aflatoxin A<sup>(69)</sup>. Traditionally, rosemary was used in renal colic as an antispasmodic<sup>(70)</sup>.

As for cat's claw (CC), is a supplement in the treatment of many human disorders including inflammations, cancer and infections<sup>(11)</sup>. It has immune-stimulating and antioxidant properties, probably related to the high concentration of flavonoids that act against reactive oxygen species (ROS), decreasing the oxidative stress in the inflammatory process, thus can improve organ functions. CC contains several alkaloids that are responsible for its medical effects, as well as tannins and various phytochemicals<sup>(71)</sup>. The chemotype of the plant determines the dominant type of alkaloid it produces, and thus its properties in vivo. One chemotype has roots which produce mostly the pentacyclic alkaloids that are responsible for the immune-strengthening effects desired by most consumers. The second chemotype produces tetracyclic oxindole alkaloids known as rhynchophylline and isorhynchophylline which counteract the immune-strengthening actions of the pentacyclic alkaloids, and reduces the speed and force of the heart's contraction<sup>(72)</sup>.

#### Conclusion:

From the results of the present study, it can be concluded that hops, rosemary and cat's claw herbs are useful natural products that can alleviate the hepatotoxicity resulted from DMBA hydrocarbon exposure. Rosemary was

the most effective then hops and cat's claw. More studies are needed.

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