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Utilization of the Aqueous Mulberry (*Morus alba*. L.) Leaf Decoction (AMLD) For Treating the DMBA Induced Hepatotoxicity and Free-Radical Damage in Norwegian Rat, *Rattus norvegicus* (L).

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ABSTRACT

The aqueous mulberry (*Morus alba* L.) leaf decoction (AMLD) is well documented for antioxidant activity. The present attempt deals with histological and biochemical evaluation of aqueous mulberry (*Morus alba* L.) leaf decoction (AMLD) for protective influence in the 7,12-Dimethylbenz[a]anthracene (DMBA) induced liver damage. The 7,12-Dimethylbenz[a]anthracene (DMBA) is acting as immuno suppressor and it serves as a tumor initiator. The promotion of cancer or tumor is possible through the treatments of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in some models of two-stage carcinogenesis. This TPA allows greatly accelerated rate of growth of tumor. Forty Norwegian Rats (*Rattus norvegicus* L) were divided into four groups, each group with ten individuals. The groups incude: 1) Untreated control group – received oral corn oil; 2) DMBA treated group –received DMBA was orally 335 mg/kg in the corn oil solution; 3) Aqueous Mulberry Leaf Decoction (AMLD) Treated group – received 100 mg AMLD/kg/day was orally, at every 24 hours for 7 days and 4) The group treated with DMBA followed by AMLD (DMBA + AMLD). All animals rats sacrificed at the end of experiment. Bioassays of Superoxide dismutases (SOD), glutathione peroxidase (GPX), nitric oxide (NO); myeloperoxidase (MPO); aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were carried in serum and liver tissue. The histopathological study on liver tissue was evaluated with Hematoxilin & Eosin stains. Biochemical studies were found that, ALT, AST, NO, MPO in serum and NO, MPO in liver tissue significantly higher in DMBA group, compared to control group ($P < 0.001$). In Group AMLD + DMBA, serum AST, ALT, NO, MPO levels were significantly lower ($P < 0.01$), and both serum and tissue SOD activities were found significantly higher, compared to DMBA group ($P < 0.001$). Severe dilation; moderate disruption of the radial alignment of hepatocyte cytoplasm; inflammation around central vein and portal region were observed as DMBA induced histopathological changes in liver tissue. In rats receiving both DMBA and AMLD, the DMBA induced changes accounted for less sinusoidal dilatation, vacuolization in the hepatocyte cytoplasm and the inflammation around central vein and portal region ($P < 0.05$). The DMBA was found to induce liver damage by oxidative

stress mechanisms. Aqueous Mulberry Leaf Decoction (AMLD) was reported to reduce the oxidative stress by inducing antioxidant mechanisms, thereby showing protective effect against DMBA induced liver damage.
Keywords : DMBA, Oxidative Stress, Antioxidants, Histopathology, Hepatotoxicity, AMLD

I. INTRODUCTION

The cancer is distinguished by abnormal cell growth. In real sense, cancer is a group of diseases. It involve abnormal cell growth with the potential to invade or spread to other parts of the body. A lump; abnormal bleeding; prolonged cough; weight loss (without understanding the cause) and change in bowel movement are some of the signs and symptoms of cancer. According to Miyata, *et al* (2001), the 7,12-Dimethylbenz [a] anthracene (DMBA) is an immunosuppressor and a powerful organ-specific to be used for studies in laboratory as carcinogen. The 7,12-Dimethylbenz [a] anthracene (DMBA) is widely used in number of research laboratories for the study of cancer. The 7,12-Dimethylbenz [a] anthracene (DMBA) is working as a tumor initiator. According to Sung, *et al* (2005), the treatment of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) is also responsible to induce the tumor promotion. This 12-*O*-tetradecanoylphorbol-13-acetate (TPA) allows for a greatly accelerated rate of tumor growth, making many cancer studies possible.

The biochemical components in the leaves of mulberry, *Morus alba* (L) deserve significant potentials. They serve a lot to orchestrate the progression of life cycle of lepidepteran insects like silkworm, *Bombyx mori* (L). The leaves of mulberry, *Morus alba* (L) are not only used for feeding the larval instars of silkworm, *Bombyx mori* (L), but also used as food material for livestock (Cattle, goat etc.). The use of leaves of mulberry, *Morus alba* (L) for feeding other than the silkworm larvae is a regular practice in the areas where dry seasons restrict the availability of ground vegetation. As a herbal medicine, in China, the fruits of mulberry, *Morus alba* (L) are used to treat the prematurely grey hair, to tonify the blood and to treat constipation and human diabetes. Zhang *et al.* (2009) reported the Moracin –M, Steppogenin–4'– O–beta-D–glucoside and mulberroside- the novel compounds of mulberry, *Morus alba* (L) for hypoglycemic effects. Naowaboot, *et al*., (2009) studied the effect of Ethanolic extract of leaves of mulberry, *Morus alba* (L) on chronic diabetic rats and observed anti-hyperglycemic, antioxidant and antiglycation activity. Cancer induction is distinguished by involvement of oxidative stress in the cells.

Hyperglycemia is the significant feature of the disease diabetes in human being. The oxidative stress induction is the prime concern with diabetic hyperglycemia. This oxidative stress leads to microvascular and macrovascular complications. In one of the recent attempt, **VBK (2018),** the streptozotocin induced diabetic male brown rats (*Rattus norvegicus* L.) were divided into five groups, each with 15 individuals. The rats of Group-I served as Normal Healthy group. The Group-II served as streptozotocin induced diabetic rats of solvent treated group. The Group-III served as streptozotocin induced diabetic rats treated with 200 mg/kg decoction of leaves of mulberry, *Morus alba* (L). The Group-IV served as streptozotocin induced diabetic rats treated with 400 mg/kg decoction of leaves of mulberry, *Morus alba* (L). The rats in Group- V were treated with glibenclamide (glyburide) (0.5 mg/kg). All the groups were maintained for three weeks. The blood samples were subjected for bioassays of After 3 weeks, blood samples serum glucose, urea and creatinine. For

the purpose to confirm the oxidative damage; attempts on lipid peroxidation and histopathology were conducted. Untreated diabetic rats were found with significant increase in serum glucose, urea and creatinine. Significant rise in lipid peroxidation with a glomerular atrophy and necrotic tubular epithelium in the renal tissue was reported in addition. The rise in serum glucose, urea and creatinine was found ameliorated through the decoction of leaves of mulberry, *Morus alba* (L). The results of the attempt are suggesting that, leaves of mulberry, *Morus alba* (L) are going to serve as effective nutritional supplement to prevent complications of diabetes. High intake of plant derived food material is well documented for low or no risk of cancer and other chronic diseases in man. In this regard, the plant derived polyphenols deserve manifold biological roles. Therefore, the plant derived polyphenols have received substantial attention (Stagos, *et al*, 2012; Rodriguez, *et al*, 2013; Devi, *et al*, 2015; Russo, *et al*, 2016). The plant derived polyphenols are generally present in the fruits; vegetables and tea. The leaves of mulberry, *Morus alba* (L) are documented for the contents like rich in polyphenols such as quercetin and caffeic acid (Chan, *et al*, 2013). The leaves of mulberry, *Morus alba* (L) are used as a traditional medicine for the treatment of several metabolic diseases like: dyslipidemia, diabetes, fatty liver disease, and hypertension (Flores, *et al*, 2012; Ou, *et al*, 2011; Kobayashi, *et al*, 2010). Earlier attempts revealed that, the aqueous decoction of mulberry leaves exert nephroprotective influence in hyperglycemia-induced oxidative stress in brown rat *Rattus norvegicus* (L) (Vitthalrao Bhimasha Khyade, 2018). Chan, *et al* (2010) and Yang, *et al*, 2011) demonstrated effective inhibition; proliferation and migration of vascular smooth muscle cells, improve vascular endothelial function, and reduce atheroma burden. Ann *et al*. (2015) reported for controlling the obesity-related fatty liver disease through their regulation of hepatic lipid metabolism, fibrosis, and the antioxidant defense system (Ann, *et al*, 2015). Because of the etiological link between obesity and liver cancer, plant polyphenols with their antioxidant and antiinflammatory properties have drawn increasing interest for their possible role in chemoprevention. However, little is known about the effect of plant polyphenols on the progression of adipocyte-induced HCC progression. The cancer induction and its subsequent development, and associated molecular mechanism is becoming increasingly clear (Lahiri. *et al*., 1999 and Ames *et al*., 1995).

Aspartate aminotransferases (AST) and alanine aminotransferases (ALT) are intracellular aminotransferase enzymes, present in liver cells. After cell death or damage in liver cells, they are released into the circulation. Increased serum transaminases translate a susceptibility to liver damage (Andreoli, *et al* , 1995). Myeloperoxidase (MPO) is the most abundant protein in neutrophils, catalyzes the conversion of hydrogen peroxide and chloride ions into hypochlorous acid. It plays a role in down regulating the inflammatory response (Klebanoff,1999). Superoxide dismutase (SOD) is regarded as the first line of defense against the detrimental effects of molecular oxygen radicals in cells. Superoxide is a crucial source of hydroperoxides and free radicals. The activity of SOD inhibits lipid peroxidation by catalyzing the conversion of superoxides into hydrogen peroxide and oxygen. The SOD protects the cells from superoxide toxicity via removing superoxide free radicals (Beyer, *et al* ,1991). Endogenous nitric oxide (NO) is formed from the amino acid L-arginine with nitric oxide synthase (NOS) enzyme. Increasing the level of NO has a crucial role in the modulation of oxidative stress and tissue damage (Tutanc, *et al* ,2012). It was reported that oxidative stress results in the increase of the activity of NO synthase, as a consequence to the elevation of NO release (Peresleni, *et al* ,1996). Glutathione peroxidase (GPx) is a crucial selenocysteine-containing enzyme, which catalyzes the reduction of hydroperoxides, including hydrogen

peroxide, by reduced glutathione and functions to protect the cells from oxidative damage (Arthur, 2000).The attempt aimed to evaluate the antioxidant and protective efficacies of silibinin against DMBA induced hepatotoxicity in rats by evaluation of NO, MPO, SOD, GPx, AST, ALT and histological values.

II. METHODS AND MATERIAL

The study was carried through the steps, which include: Preparation of Aqueous Mulberry Leaf Decoction (AMLD); Rearing of brown rat, *Rattus norwegicus* (L), the experimental animals; Design of Experiment through Grouping the experimental animals; Processing for the assay sample preparation (Serum Assay Sample and Liver tissue homogenate); Biochemical Analysis; Histochemical studies and Statistical analysis.

(A). Preparation of Aqueous Mulberry Leaf Decoction (AMLD):

The leaves of mulberry, *Morus alba* (L,) were collected from moriculture unit (mulberry garden) of "Dr. APIS", Malegaon Sheti Farm, Agricultural Development Trust Baramati, Shardanagar, (Malegaon Khurd) Post Box No - 35, Baramati, Pune 413 115, Maharashtra, India. The leaves were identified, confirmed through expertise support from the "Dr. APIS" Laboratory (Shrikrupa Residence, Teachers Society, Malegaon Colony, Baramati Dist. Pune – 413115 Maharashtra, India). The leaves of mulberry, *Morus alba* (L,) were kept in shade, dry and ventilated place, protected from light. After confirmation about complete dried condition, the leaves were processed for preparation of powder through the use of domestic mixture. The fresh decoction was prepared daily. Twenty grams of mulberry leaves were processed for boiling in one liter of distilled water for ten minutes. The content was allowed for cooling. After reaching room temperature, the content was filtered and the filtrate was equalized to the volume to reach one liter by addition of distilled water. The strength of decoction was 20 g / L. The resulting content was used for experimental use in drinkable form.

(B). Rearing of Brown rat, *Rattus norwegicus* (L), the Experimental Animals:

For the present attempt on utilization of the aqueous mulberry (*Morus alba*. L.) leaf decoction (AMLD) for treating the DMBA induced hepatotoxicity and free-radical damage in Norwegian rat, *Rattus norvegicus* (L), fifty adult female (12 week-old) Brown rats (*Rattus norvegicus* L) (Dr. APIS Laboratory), weighing 170 - 220 g were procured from the

Department of Zoology, Savitribai Phule Pune University. The adult female rats were housed in quiet cages (20 - 25°C; 50 - 60% relative humidity). They were kept in laboratory with a condition of "12 hour light/dark cycle (7 a.m. - 7 p.m.)". They were fed with a commercial standard rat diet (Abaliogu YemSanayi, Denizli, Turkey) and water ad libitum. All animal procedures were approved by the Animal Care and Use Protocol (Department of Zooloy, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Baramati).

(C). Design of Experiment Through Grouping the Experimental Animals:

Total fifty adult female brown Norwegian rat, *Rattus norvegicus* (L) (12 week-old) Brown rats weighing 170 - 220 g were procured from the Department of Zoology, Savitribai Phule Pune University. They were housed in quiet cages (20 - 25°C; 50 - 60% relative humidity). They were kept in laboratory with a condition of "12 hour light/dark cycle (7 a.m. - 7 p.m.)". They were fed with a commercial standard rat diet (Abaliogu Yem Sanayi, Denizli, Turkey) and water ad libitum. All animal procedures were approved by the Animal Care and Use Protocol (Department of Zooloy, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Baramati). The experimental animals were divided into four groups, each with ten individuals. Remaining ten individuals of experimental animals were maintained as reservoir. The indivuals of group I were served as untreated control group. The individuals of this untreated control group received 0.3 ml corn oil daily orally.

The experimental animals in the attempt were randomly divided into four groups. Each group was with ten individuals. The four groups of experimental animals in the attempt are as below: -

-Group 1: Control group (n = 10). The rats in this group were supplied with 0.3 mL corn oil orally.

- Group 2: DMBA group (n = 10). The rats in this group were supplied with 7,12-Dimethylbenz[a]anthracene (DMBA). The single dosage of DMBA at the rate 335 mg/kg of body weight was selected. The DMBA was given along with corn oil (Shah and Iqbal,2010;Yilmaz, *et al* , 2012 and Teimouri, 2007);

- Group 3: AMLD group (n = 10). The rats in this group were supplied with aqueous mulberry leaf decoction (AMLD). The aqueous mulberry leaf decoction (AMLD) was given orally, 100 mg/kg/day every twenty four hours. This AMLD treatment was continued for seven days.

- Group 4: DMBA + AMLD group (n = 10). The rats in this group were the recipient of single dosage of DMBA. The strength of DMBA dosage was of 335 mg/kg Body Weight. The rats in this group were also supplied with aqueous mulberry leaf decoction (AMLD). The number of dosages of AMLD were seven. Each dosage of AMLD was after every twenty four hours. The strength of each dosage of AMLD was 100 mg/kg/day. The AMLD treatment was oral, at every twenty four hours and it was for total seven days.

(D). Processing for the Assay Sample Preparation:

At the end of the schedule of seven days of treatment, experimental animals were anesthetized. This anaesthetization was carried through two intra-periteoneal injections. The first intraperitoneal injection was of ketamine (60 mg/kg). The second intraperitoneal injection belong to xylazine (6 mg/kg). The gap between the two injections was fifteen minutes. Through the use of sterile tubes, the blood samples intracardiac were collected.

Preparation of Serum Sample **For Biochemical Analysis**:

For the purpose of bioassay of the level of serum ALT, AST, MPO, NO, SOD and GPx, the blood samples were processed for centrifugation and serum preparation. Each blood sample was allowed for centrifugation at 2000 × G for 15 minutes, at 4°C. The

serum use to appear as the top yellow layer in centrifugation tube. This top yellow serum layer was pipetted out. Care was taken for keeping the white buffy layer as it was (Serum was collected without disturbing the white buffy layer). Liver tissue from individual experimental animal in each group was removed immediately. The liver tissue was washed with phosphate buffer solution (PBS) (pH = 7.4). The liver tissue was then frozen promptly in a deep freezer for biochemical analysis. All samples were protected through keeping them under -80°C until analysis.

Preparation of Liver Tissue Assay Sample for Biochemical Analysis:

The experimental animals were anesthetized. This anaesthetization was carried through two intra-periteoneal injections. The first intraperitoneal injection was of ketamine (60 mg/kg). The second intraperitoneal injection belong to xylazine (6 mg/kg). The gap between the two injections was fifteen minutes. The experimental animals were dissected. The liver from each individual was removed aseptically. The liver was processed for "Flash-freezing in foil packets using liquid nitrogen" and stored at –80°C. For the purpose to prepare homogenate, the liver sample was crushed on dry ice and stored in a prechilled 5 ml culture tube. Care was taken for the addition of about 100 µg tissue (enough to almost cover round portion of a 5 ml culture tube) to a new chilled tube. Addition of 1 ml PBS with 10 µl protease inhibitors (Sigma Cat. no. P-8340) was made. This solution then allowed for chilling using wet ice. The content was processed for homogenization at low speed for near about twenty seconds. The entire system was kept cold and tissue sample was kept on wet ice. The tissue sample was then transferred into 1.7 ml microcentrifuge tubes. It was the processed for centrifugation at 14,000 x g at 4°C for 15 minutes. The aliquot supernatant was separated and used as assay sample.

(E). Biochemical Analysis:

(1). Bioassay of Aspartate Aminotransferase and Alanine Aminotransferase Activities:

The bioassay of activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase Activities (ALT) were carried out using the method of Kori-Siakpere Ovie, *et al* (2010). The results were expressed as units per liter (U/L).

Plasma alanine aminotransferase and aspartate aminotransferase were determined colorimetrically using commercial diagnostic kits (Dialab Producktion, Austria) using a spectrophotometer (Spectrumlab 21A, Lenjguang Tech, China).

Plasma alanine aminotransferase and aspartate aminotransferase were measured based upon International Federation of Clinical Chemistry (IFCC) recommendations by kinetic decreasing methods.

Plasma aspartate aminotransferase was measured based on the oxido-reductive process of $NADH/NAD^+$ in the presence of oxalo- acetate and the resulting decrease in absorbance at 340 nm being directly proportional to the aspartate aminotransferase in the sample (U/L).

Plasma alanine aminotransferase was similarly measured based on the oxido-reductive process of $NADH/NAD^+$ in the presence of pyruvate and the resulting decrease in absorbance at 340 nm being directly proportional to the alanine aminotransferase in the sample (U/L).

(2). Bioassay of Superoxide Dismutase Activity:

Determination of Superoxide Dismutase (SOD) Activity was determined via Cayman's Superoxide Dismutase assay kit (Cayman Chemical Co., Ann Arbor, MI, USA) in Bio-Tek ELx-800 (Winooski, USA). The detection of superoxide radicals were generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme required to exhibit 50% dismutation of the superoxide

radical. The results were expressed as units per mg protein (U/mg) tissue, for liver tissue, and units per milliliter (U/mL) for serum. The dynamic range of the kits is 0.005 - 0.05 U/mL SOD. Recommended by the company for measuring formulation, the SOD was calculated by applying SOD values.

(3). Bioassay of Glutathione Peroxidase Activity:

The tissue was homogenized in 5-10 mL cold buffer (50 mMTris-HCl, pH 7.5, 5 mM EDTA and 1 mM DTT (Dithiothreitol) per tissue. Then, it was centrifuged at 10000 × G for 15 minutes, at 4°C. The supernatant was removed after centrifugation. The blood was centrifuged at 700 - 1000 × G for 10 minutes, at 4°C. The serum was removed. The GPx activity was measured in liver tissue and serum samples. The GPx activity was determined via Cayman's GPx assay kit (Cayman Chemical Co., Ann Arbor, MI, USA) in Bio-Tek ELx-800. The GPx activity was measured indirectly by a coupled reaction with glutathione reductase. The oxidized glutathione was produced upon reduction of hydroperoxide by GPx. The results were expressed as units per mg protein (U/mg) tissue, for liver tissue, and units per milliliter (U/mL), for serum. The dynamic range of the assay is only limited by the accuracy of the absorbance measurement.

(4). Bioassay of Nitric Oxide (*NO*) Level:

Determination of Nitric Oxide Level The tissue was homogenized in PBS (pH 7.4) and centrifuged at 10000 × G for 20 minutes to create the supernatant. Total NO assay was performed by spectrophotometry at 540 nm using nitrate/nitrite colorimetric assay kit (Cayman, Ann Arbor, Michigan USA) in Bio-Tek ELx-800. The assay was based on nitrate and nitrite determinations. The nitrate and nitrite are the stable end products of the reaction of NO with molecular oxygen. The total accumulation of nitrate and nitrite in serum and liver tissue was measured. The results were expressed as μm/g protein.

(5). Bioassay of *Myeloperoxidase* (*MPO*) Activity:

The quantitative detection of MPO was used by an enzyme-linked immunosorbent assay (ELISA) kit (MPO Instant Elisa, eBioscience, Vienna, Austria) in Bio-TekELx- 800. The results were expressed as ng/mL protein.

(F). Histopathology of Liver Tissue:

The liver tissue was also removed for histopathological investigation. The specimens were fixed in 10% formalin subsequent overnight and then were dehydrated by immersion in a series of alcohol solutions of various concentrations, cleared in xylene and paraffin embedded tissue sections. The tissue samples were then infiltrated with paraffin as blocks, sectioned (5 μm-thick slides). The prepared samples were examined under a light microscope according to the severity of the lesions modified from Yehia, *et al* (2007). Each parameter was scored between 0 and 3 (0: normal, 1: mild, 2: moderate and 3: severe) and according to the point total, lesions were classified into three grades (grade 1: 1-5 points, grade 2: 6 - 10 points and grade 3: 11-15 points) (Andreoli, *et al* , 1995 and Klebanoff,1999).

Six parameters of liver damage were evaluated:

1. Sinusoidal dilatation;
2. Distortion radial alignment around central vein;
3. Vacuolization in hepatocytes;
4. Inflammation in the portal area and around central vein;
5. Hepatocellular necrosis;
6. Eosinophils infiltration of in the periportal field or around central vein.

(G). Statistical Analysis:

Data were analyzed using a commercially available statistics software package (SPSS Statistics for Windows, Version 20.0. IBM Corp., Armonk, NY, USA). All data were presented as the mean ± SD for comparisons. Comparisons between groups were

performed using the KruskalWallis analysis of variance for unpaired comparisons, followed by the Mann Whitney U test. The P < 0.05 was considered significant.

III.RESULTS AND DISCSSION

The results on the attempt on utilization of the aqueous mulberry (*Morus alba*. L.) leaf decoction (AMLD) for treating the DMBA induced hepatotoxicity and free-radical damage in Norwegian Rat, *Rattus norvegicus* (L) are summarized in tables (table- 1 and 2) and presented in figures (Fig.1,2 and 3).

(I). Biochemical Results

The velocity of biochemical reaction catalyzed by the enzymes like asparateamino transferase (AST) and alanine aminotransferase (ALT) in the serum sample of the experimental animals (Rat, *Rattus norvegicus* L.) in the untreated control group was found recorded 108.71 **(± 23.604) and** 55.681 (±13.173) units respectively (Table-1).

The velocity of biochemical reaction catalyzed by the enzymes like asparateamino transferase (AST) and alanine aminotransferase (ALT) in the serum sample of the experimental animals (Rat, *Rattus norvegicus* L.) in the DMBA treated group was found recorded 80.711 (± 14.039) **and** 90.906 (± 10.281) units respectively (Table-1).

Table – 1: Influence of Aqueous Mulberry Leaf Decoction on Biochemical Parameters of Serum in DMBA Induced Hepatotoxicity and Free-Radical Damage in Norwegian Rat, *Rattus norvegicus* (L).

Groups	AST, U/L	ALT, U/L	SOD, U/mL	GPx, U/mL	NO, µm/g	MPO, ng/mL
Control group	**108.71 (± 23.604)**	55.681 (±13.173)	0.102 (± 0.021)	3.514 (± 0.989)	1.445 (± 0.739)	1.527 (± 1.031)
DMBA group	80.711 (± 14.039)	90.906 (± 10.281)	0.091 (± 0.023)	3.748 (± 2.798)	7.201 (± 2.759)	4.786 (± 1.617)
AMLD group	91.405 (± 11.362)	45.328 (± 3.22)	3.726 (± 2.605)	8.029 (± 2.706)	1.272 (± 0.786)	1.211 (± 0.514)
DMBA + AMLD group	106.05 (± 23.573)	54.792 (± 10.089)	0.523 (± 0.345)	7.545 (± 2.456)	2.565 (± 1.364)	0.818 (± 0.334)

Abbreviations:

AMLD: Aqueous Mulberry Leaf Decoction

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GPx: Glutathione peroxidase; MPO: Myeloperoxidase; NO: Nitric oxide; SOD: Superoxide dismutases.

-Data is presented as Mean ± SD for n = 8.
- P < 0.05 SOD, DI + AMLD compared with DMBA group.
- P < 0.05 NO, DI + AMLD compared with DMBA group.
- P < 0.05 MPO, DI + AMLD compared with DMBA group.

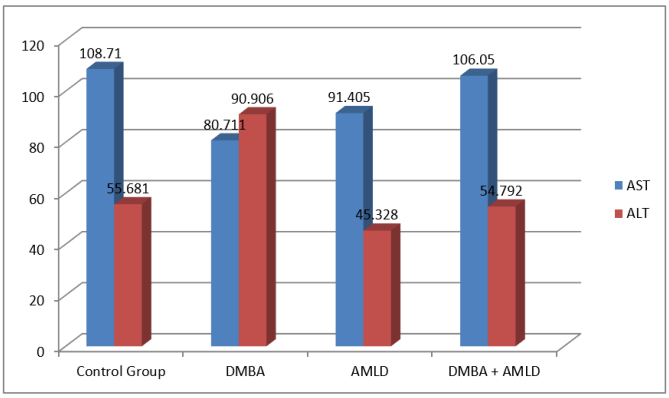

Fig.– 1: Serum Aspartate Aminotransferase and Alanine Aminotransferase Activities in Norwegian Rat, *Rattus norvegicus* (L) receipient of separate DMBA; separate Aqueous Mulberry Leaf Decoction (AMLD) and both DMBA; Aqueous Mulberry Leaf Decoction (AMLD) together.

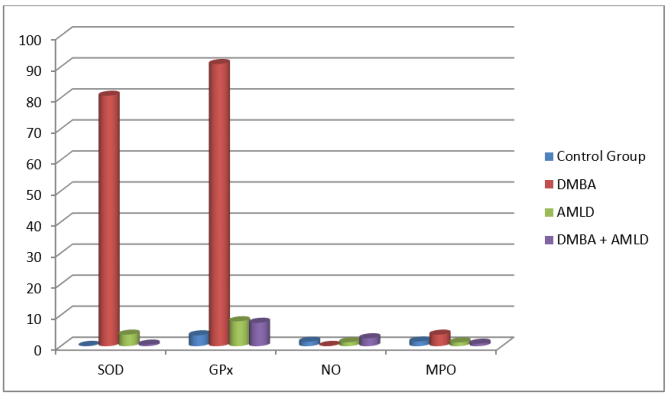

Fig.– 2: Serum Superoxide Dismutase (SOD); Glutathione Peroxidase (GPx); Nitric Oxide (NO) and *Myeloperoxidase* (MPO) Activities in Norwegian Rat, *Rattus norvegicus* (L) receipient of separate DMBA; separate Aqueous Mulberry Leaf Decoction (AMLD) and both DMBA; Aqueous Mulberry Leaf Decoction (AMLD) together.

Table 2. Influence of Aqueous Mulberry Leaf Decoction on Biochemical Parameteres of Liver in DMBA Induced Hepatotoxicity and Free-Radical Damage in Norwegian Rat, *Rattus norvegicus* (L).

Groups	GPx, U/mg	SOD, U/mg	NO, µm/g	MPO, ng/mL
Control group	0.897 (± 0.213)	1.615 (± 0.475)	2.868 (± 1.859)	3.546 (± 0.823)
DMBA group	0.768 (± 0.232)	2.726 (± 0.863)	9.546 (± 2.734)	7.615 (± 3.354)
AMLD group	0.968 (± 0.293)	3.876 (± 1.493)	2.223 (± 0.011)	4.605 (± 0.571)
DMBA+ AMLD group	0.182 (± 0.112)	2.843 (± 0.839)	5.958 (± 1.153) c	3.768 (± 0.567) d

IV. METHODS AND MATERIAL

-Abbreviations:

AMLD: Aqueous Mulberry Leaf Decoction

GPX: Glutathione peroxidase; MPO: Myeloperoxidase; NO: Nitric oxide;

SOD: Superoxide dismutases.

-Data is presented as Mean ± SD for n = 8.

-P < 0.05 NO, DI + AMLD, compared with DMBA group.

-P < 0.05 MPO, DI + AMLD, compared with DMBA group.

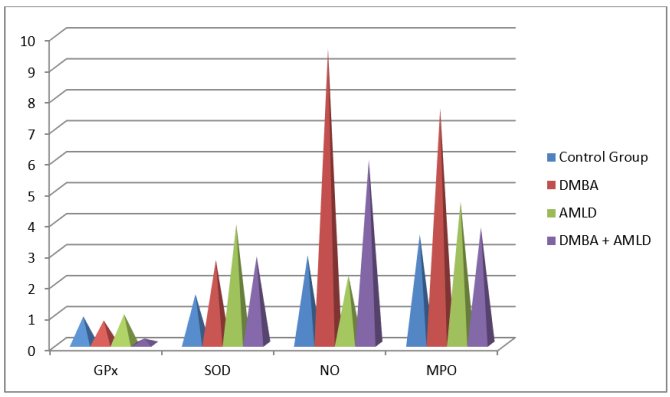

Fig.– 3: Superoxide Dismutase (SOD); Glutathione Peroxidase (GPx); Nitric Oxide (NO) and *Myeloperoxidase* (MPO) Activities in the liver homogenate of Norwegian Rat, *Rattus norvegicus* (L)

receipient of separate DMBA; separate Aqueous Mulberry Leaf Decoction (AMLD) and both DMBA; Aqueous Mulberry Leaf Decoction (AMLD) together.

The velocity of biochemical reaction catalyzed by the enzymes like asparateamino transferase (AST) and alanine aminotransferase (ALT) in the serum sample of the experimental animals (Rat, *Rattus norvegicus* L.) in the aqueous mulberry leaf decoction (AMLD) treated group was found recorded 91.405 (± 11.362) and 45.328 (± 3.22) units respectively (Table-1).

The velocity of biochemical reaction catalyzed by the enzymes like asparateamino transferase (AST) and alanine aminotransferase (ALT) in the serum sample of the experimental animals (Rat, *Rattus norvegicus* L.) in the group treated with both DMBA and AMLD (DMBA + AMLD) was found recorded 106.05 (± 23.573) and 54.792 (± 10.089) units respectively(Table-1).

The velocity of biochemical reaction catalyzed by the enzymes like Superoxide dismutase (SOD); Glutathione peroxidase (GPx) and *Myeloperoxidase* (*MPO*) in the assay sample of serum of the experimental animals (Rat, *Rattus norvegicus* L.) in the untreated control group was found recorded 0.102 (± 0.021); 3.514 (± 0.989) and 1.527 (± 1.031) units respectively (Table-1).

The velocity of biochemical reaction catalyzed by the enzymes like Superoxide dismutase (SOD); Glutathione peroxidase (GPx) and *Myeloperoxidase* (*MPO*) in the assay sample of liver serum of the experimental animals (Rat, *Rattus norvegicus* L.) in the DMBA treated group was found recorded 0.091 (± 0.023); 3.748 (± 2.798) and 4.786 (± 1.617) units respectively (Table-1).

The velocity of biochemical reaction catalyzed by the enzymes like Superoxide dismutase (SOD); Glutathione peroxidase (GPx) and *Myeloperoxidase* (*MPO*) in the assay sample of serum of the experimental animals (Rat, *Rattus norvegicus* L.) in the group treated with aqueous mulberry leaf decoction (AMLD) was found recorded 3.726 (± 2.605) ; 8.029 (± 2.706) and 1.211 (± 0.514) units respectively (Table-1).

The velocity of biochemical reaction catalyzed by the enzymes like Superoxide dismutase (SOD); Glutathione peroxidase (GPx) and *Myeloperoxidase* (*MPO*) in the assay sample of serum of the experimental animals (Rat, *Rattus norvegicus* L.) in the group treated with both DMBA and aqueous mulberry leaf decoction (DMBA + AMLD) was found recorded 0.523 (± 0.345); 7.545 (± 2.456) and 0.818 (± 0.334) units respectively (Table-1).

The velocity of biochemical reaction catalyzed by the enzymes like Superoxide dismutase (SOD); Glutathione peroxidase (GPx) and *Myeloperoxidase* (*MPO*) in the assay sample of liver homogenate of the experimental animals (Rat, *Rattus norvegicus* L.) in the untreated control group was found recorded 1.615 (± 0.475); 0.897 (± 0.213) and 3.546 (± 0.823) units respectively (Table-2).

The velocity of biochemical reaction catalyzed by the enzymes like Superoxide dismutase (SOD); Glutathione peroxidase (GPx) and *Myeloperoxidase* (*MPO*) in the assay sample of liver homogenate of the experimental animals (Rat, *Rattus norvegicus* L.) in the DMBA treated group was found recorded 2.726 (± 0.863) and 7.615 (± 3.354) units respectively (Table-2).

The velocity of biochemical reaction catalyzed by the enzymes like Superoxide dismutase (SOD); Glutathione peroxidase (GPx) and *Myeloperoxidase* (*MPO*) in the assay sample of liver homogenate of the experimental animals (Rat, *Rattus norvegicus* L.) in the group treated with aqueous mulberry leaf decoction (AMLD) was found recorded 3.876 (± 1.493) ; 0.968 (± 0.293) and 4.605 (± 0.571) units respectively (Table-2).

The velocity of biochemical reaction catalyzed by the enzymes like Superoxide dismutase (SOD); Glutathione peroxidase (GPx) and *Myeloperoxidase* (*MPO*) in the assay sample of liver homogenate of the experimental animals (Rat, *Rattus norvegicus* L.) in the group treated with both DMBA and aqueous mulberry leaf decoction (DMBA + AMLD) was found recorded 2.843 (± 0.839); 0.182 (± 0.112) and 3.768 (± 0.567) units respectively (Table-2).

The levels of nitric oxide in the serum and liver homogenate of the experimental animals (*Rattus norvegicus* L.) of untreated control group were found recorded 1.445 (± 0.739) and 2.868 (± 1.859) respectively (Table-1 and 2).

The levels of nitric oxide in the serum and liver homogenate of the experimental animals (*Rattus norvegicus* L.) of DMBA treated group were found recorded 7.201 (± 2.759) and 9.546 (± 2.734) respectively (Table-1 and 2).

The levels of nitric oxide in the serum and liver homogenate of the experimental animals (*Rattus norvegicus* L.) of AMLD treated were found recorded 1.272 (± 0.786) and 2.223 (± 0.011) respectively (Table-1 and 2).

The levels of nitric oxide in the serum and liver homogenate of the experimental animals (*Rattus norvegicus* L.) of belong to the group treated with both DMBA and AMLD (DMBA + AMLD) were found recorded 2.565 (± 1.364) and 5.958 (± 1.153) respectively (Table-1 and 2).

The levels of NO and MPO in serum and liver tissue were found to be significantly increased in the treated group, compared to control group ($P < 0.0001$). The activity of ALT and AST were found to be significantly increased in treated group, compared to control group ($P < 0.001$). The levels of NO and MPO in serum were found to be significantly decreased in DMBA + AMLD group, compared with DI group ($P < 0.01$ and $P < 0.001$). When comparing DMBA + AMLD group to DMBA group the activities of AST and ALT were found decreased. There was no significant statistical difference between the tissue or serum GPx activities, for all groups. The serum and tissue SOD activity was found increased in DMBA + AMLD group, when compared to DI group ($P < 0.001$). The present attempt suggest that DMBA induced a significant liver damage. The aqueous mulberry leaf decoction (AMLD) was reported to reduce the levels of AST, ALT, NO, MPO in AMLD + DMBA group, compared with DI group. Aqueous Mulberry Leaf Decoction (AMLD), given to rats with DMBA, showed a significant protective activity against liver damage induced by DMBA. In addition, the level of SOD in serum and liver tissue increased via in Aqueous Mulberry Leaf Decoction (AMLD) in "AMLD + DMBA group".

V. HISTOPATHOLOGICAL RESULTS

Hepatocytes of control group (Figure 1 A) and silibinin group (Figure 1 B) were observed to have a normal structure (Figure 1). It was determined histopathologically that the liver tissue intoxicated by DMBA was significantly damaged (Grade 3). In histopathological examination, rats administered DMBA showed severe sinusoidal dilatation, moderate disrupt radial alignment of hepatocytes, severe vacuolization of hepatocyte cytoplasm, and centrilobular necrosis ($P < 0.05$) (Figure 2). In contrast, rats in DMBA + AMLD group exhibited these changes significantly, especially inflammation around the central vein and portal space ($P < 0.05$) (Figure 3). In histopathological examination, DMBA was found to cause severe sinusoidal dilatation and severe vacuolation, inflammation around the portal area and central vein and disrupted the radial alignment around the central vein in hepatocytes. The aqueous mulberry leaf decoction (AMLD) significantly reversed the DMBA-induced sinusoidal dilatation, severe vacuolization and inflammation around the central vein in hepatocytes ($P < 0.05$).

The liver is a very crucial organ for the detoxification processes and oxidative stress is thought to be a key mechanism of hepatocellular injury. Through the assemblage of great amunt of metabolite, the liver tissue is reported as the major site of DMBA metabolism (Giray, *et al* , 2011). The DMBA increased the reactive oxygen species (ROS) in liver tissue and aqueous mulberry leaf decoction (AMLD) carries out free-radical-eliminating activity and extensive antioxidant effect. Akturk, *et al* (2007) and Yilmaz, *et al* (2012), reported increase in lipid peroxidatio through DMBA. It was reported that DMBA exposure has been implicated in inducing oxidative stress increased nitrate and nitrite. The MPO, which is a peroxidase enzyme that synthetizes hypochlorous acid from H_2O_2 and chloride, plays an important role, as a powerful oxidant, which utilizes free radicals (Klebanoff, 1999; Mehta, *et al* , 2009; Klebanoff, 2005; Heinecke, *et al* , 1993). In this study, DMBA exposure could induce oxidative stress by the increased NO, MPO concentrations, which should induce membrane lipid peroxidation, resulting in liver injury. These results were correlated with previous reports of Messarah, *et al*. who showed DMBA might generate ROS (Messarah, *et al* , 2013). It was reported that Aqueous Mulberry Leaf Decoction (AMLD) reduce the hyperglycemia induced oxidative stress in brown rat, *Rattus norvegicus* (L.) (*Vitthalrao Bhimasha Khyade and Avram Hershko,* 2018). To the best knowledge of authors, the present study is the first to investigate antioxidant effects of the Aqueous Mulberry Leaf Decoction (AMLD) in DMBA induced hepatotoxicity. Oxidative stress induced by DMBA administration is also demonstrated by a highly significant increase in the activities NO and MPO and our results are in agreement with previous reports (Messarah, *et al* , 2013; Sozmen, *et al* , 2014). The DMBA affects the mitochondrial membrane transportation in rat liver (Nakagawa and Moore 1999). Diazinon (Lee, *et al* , 1991) and DMBA (Vitthalrao B. Khyade, *et al* , 2014) binds extensively to biological membranes, especially to the phospholip-ids bilayers. The contents of aqueous mulberry leaf decoction (AMLD) may be acting on the polar head group of phospholipids of the cellular membrane.

Conclusively enough, the attempt demonstrate that aqueous mulberry leaf decoction (AMLD) exerts hepatoprotective, antioxidant, free radical scavenging effects against DMBA induced hepatotoxicity. It may also be be suggested that aqueous mulberry leaf decoction (AMLD) is convenient as a therapeutic agent for the amelioration of DMBA induced hepatotoxicity. However, further studies are required in order to understand the chemical components and quantify the beneficial effects of aqueous mulberry leaf decoction (AMLD) and it's possible clinical use.

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VII.REFERENCES

[1]. Akturk, O., Demirin, H., Sutcu, R., Yilmaz, N., Koylu, H., Altuntas, I. 2006. The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat heart and ameliorating role of vitamin E and vita-min C. Cell Biol. Toxicol., 22(6):455– 61.

[2]. Alp H, Aytekin I, Esen H, Basarali K, Kul S. Effects of caffeic acid phenethyl ester, ellagic acid, sulforaphane and curcumin on diazi¬non induced damage to the lungs, liver and kidneys

in an acute toxicity rat model. KafkasUniv Vet FakDerg. 2011; 17(6):927–33.

[3]. Andreoli TE, Carpenter CCJ, Cecil RLF. (1995). Cecil Essentials of Medicine. 3rd ed Michigan: Saunders; 1995.

[4]. Ann JY, Eo H, Lim Y. (2015). Mulberry leaves (Morus alba L.) ameliorate obesity-induced hepatic lipogenesis, fibrosis, and oxidative stress in high-fat diet-fed mice. Genes Nutr 2015;10:46.

[5]. Arthur JR. The glutathione peroxidases(2000). Cell Mol Life Sci. 2000;57(13-14):1825–35.

[6]. Babita M. Sakdeo and Vitthalrao B. Khyade(2013): Effect of Moracin on DMBA – TPA induced Skin Tumor Formation in the Mice. 2013. International Journal of Advanced Biological Research Vol. 3 (4): 576 – 583. www.scienceandnature.org/IJABR.../Index%20IJABR-vol3(4).pdf

[7]. Barzaghi N, Crema F, Gatti G, Pifferi G, Perucca E (1990). "Pharmacokinetic studies on IdB 1016, a silybin- phosphatidylcholine complex, in healthy human subjects". Eur J Drug MetabPharmacokinet. 15: 333–8. PMID 2088770.

[8]. Beyer W, Imlay J, Fridovich I. (1991). Superoxide dismutases.(Prog Nucleic Acid Res Mol Biol. 1991; 40:221–53.

[9]. Bosch-Barrera J, Menendez JA (2015). "Silibinin and STAT3: A natural way of targeting transcription factors for cancer therapy". Cancer Treat. Rev. (Review). 41 (6): 540–6. doi:10.1016/j.ctrv.2015.04.008. PMID 25944486.

[10]. Cacciapuoti F, Scognamiglio A, Palumbo R, Forte R, Cacciapuoti F. Silymarin in non alcoholic fatty liver disease. World J Hepatol. 2013;5(3):109–13.

[11]. Chan KC, Ho HH, Peng CH, Lan KP, Lin MC, Chen HM, et al. Polyphenol-rich extract from mulberry leaf inhibits vascular smooth muscle cell proliferation involving up-regulation of p53 and inhibition of cyclin-dependent kinase. J Agric Food Chem 2010;58:2536-42.

[12]. Chan KC, Ho HH, Peng CH, Lan KP, Lin MC, Chen HM, et al. (2010). Polyphenol-rich extract from mulberry leaf inhibits vascular smooth muscle cell proliferation involving up-regulation op53 and inhibition of cyclin-dependent kinase. J Agric Food Chem 2010;58:2536-42.

[13]. Chan KC, Yang MY, Lin MC, Lee YJ, Chang WC, Wang CJ. (2013). Mulberry leaf extract inhibits the development of atherosclerosis in cholesterol-fed rabbits and in culturedaortic vascular smooth muscle cells. J Agric Food Chem 2013;61:2780-8.

[14]. Coppack SW. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 2001;60:349-56.

[15]. Davis-Searles P, Nakanishi Y, Nam-Cheol K, et al. (2005). "Milk Thistle and Prostate Cancer: Differential Effects of Pure Flavonolignans from Silybummarianum on Antiproliferative End Points in Human Prostate Carcinoma Cells". Cancer Research. 65(10): 4448–57. doi:10.1158/0008-5472.CAN-04-4662.

[16]. Devi KP, Rajavel T, Nabavi SF, Setzer WN, Ahmadi A, Mansouri K, et al. (2015). Hesperidin: a promising anticancer agent from nature. Ind Crop Prod 2015;76:582-9.

[17]. Erlejman AG, Verstraeten SV, Fraga CG, Oteiza PI. The interaction of flavonoids with membranes: potential determinant of flavo¬noid antioxidant effects. Free Radic Res. 2004;38 (12):1311–20.

[18]. Ezhilarasan D, Karthikeyan S, Vivekanandan P. Ameliorative effect of silibinin against N-nitrosodimethylamine-induced hepatic fi¬brosis in rats. Environ ToxicolPharmacol. 2012; 34(3):1004–13.

[19]. Flores MB, Rocha GZ, Damas-Souza DM, Osorio-Costa F,Dias MM, Ropelle ER, et al. (2012). Obesity-induced increase in tumor necrosis factor-alpha leads to development of

colon cancer in mice. Gastroenterology 2012;143:741-753

[20]. Fraschini F, Demartini G, Esposti D (2002). "Pharmacology of Silymarin". Clinical Drug Investigation. 22 (1): 51–65. doi:10.2165/00044011-200222010-00007.

[21]. Galloway T, Handy R. Immunotoxicity of organophosphorous pesticides. Ecotoxicology. 2003;12(1-4):345–63.

[22]. Gazak R, Walterova D, Kren V. Silybin and silymarin--new and emerging applications in medicine. Curr Med Chem. 2007; 14(3):315–38.

[23]. Giordano G, Afsharinejad Z, Guizzetti M, Vitalone A, Kavanagh TJ, Costa LG. Organophosphorus insecticides chlorpyrifos and diazi¬non and oxidative stress in neuronal cells in a genetic model of glutathione deficiency. ToxicolApplPharmacol. 2007;219(2-3):181–9.

[24]. Giray B, Gurbay A, Hincal F. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopuri¬nol. ToxicolLett. 2001;118(3):139–46.

[25]. Grivennikov SI, Karin M. Inflammatory cytokines in cancer: tumour necrosis factor and interleukin 6 take the stage. Ann Rheum Dis 2011;70(Suppl. 1):i104-8.

[26]. Hahn G, Lehmann HD, Kürten M, Uebel H, Vogel G (1968). "On the pharmacology and toxicology of silymarin, an antihepatotoxic active principle from Silybummarianum (L.) gaertn". Arzneimittelforschung. 18 (6): 698–704. PMID 5755807.

[27]. Harvey AE, Lashinger LM, Hursting SD. The growing challenge of obesity and cancer: an inflammatory issue. Ann N Y Acad Sci 2011;1229:45-52.

[28]. Hefetz-Sela S, Scherer PE. Adipocytes: impact on tumor growth and potential sites for therapeutic intervention. Pharmacol Ther 2013;138:197-210.

[29]. Heinecke JW, Li W, Francis GA, Goldstein JA. (1993). Tyrosyl radical gener¬ated by myeloperoxidase catalyzes the oxidative cross-linking of proteins. J Clin Invest. 1993;91(6):2866–72.

[30]. https://www.thermofisher.com/in/en/home/references/protocols/cell-and-tissue-analysis/elisa-protocol/elisa-sample-preparation-protocols/rat-liver-preparation.html

[31]. Kidd P, Head K (2005). "A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybin-phosphatidylcholine complex (Siliphos)" (PDF). Alternative Medicine Review. 10 (3): 193–203. PMID 16164374. Retrieved 2010-12-14.

[32]. Klebanoff SJ. Myeloperoxidase. ProcAssoc Am Physicians. 1999; 111(5):383–9.

[33]. Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol. 2005;77(5):598–625.

[34]. Kobayashi Y, Miyazawa M, Kamei A, Abe K, Kojima T. (2010). Ameliorative effects of mulberry (Morus alba L.) leaves on hyperlipidemia in rats fed a high-fat diet: induction of fatty acid oxidation, inhibition of lipogenesis, and suppression of oxidative stress. Biosci Biotechnol Biochem 2010;74:2385-95.

[35]. Kori-Siakpere Ovie; Ikomi Robert Bemigho and Ogbe Martin Gbemi (2010). Variations in alanine aminotransferase and aspartate aminotransferase activities in African catfish: Clarias gariepinus (Burchell, 1822) at different sublethal concentrations of potassium permanganate. Scientific Research and Essays Vol. 5(12), pp. 1501-1505, 18 June, 2010. ISSN 1992-2248 ©2010 Academic Journals. http://www.academicjournals.org/SRE

[36]. Kosina P, Kren V, Gebhardt R, Grambal F, Ulrichová J, Walterová D (2002). "Antioxidant properties of silybin glycosides". PhytotherapyResearch : PTR. 16 Suppl 1: S33–9. doi:10.1002/ptr.796. PMID 11933137.

[37]. Lazarević-Pašti T, Čolović M, Savić J, Momić T, Vasić V. Oxidation of diazinon and malathion

by myeloperoxidase. Pesticide Biochem Physiol. 2011;100(2):140–4.

[38]. Lee AG, Malcolm East J, Balgavy P. (1991). Interactions of insecticides with biological membranes. Pesticide Sci. 1991;32(3):317–27.

[39]. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inf 2013:2013.

[40]. Malley CO, Pidgeon GP. The mTOR pathway in obesity driven gastrointestinal cancers: potential targets and clinical trials.BBA Clin 2016;5:29-40.

[41]. Mehta A, Verma RS, Srivastava N. (2009). Chlorpyrifos induced altera¬tions in the levels of hydrogen peroxide, nitrate and nitrite in rat brain and liver. Pesticide Biochem Physiol. 2009;94(2-3):55–9.

[42]. Messarah M, Amamra W, Boumendjel A, Barkat L, Bouasla I, Ab¬dennour C, et al. Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythro¬cytes. ToxicolInd Health. 2013;29(1):77–88.

[43]. Miyata M; Furukawa M; Takahashi K; Gonzalez FJ; Yamazoe Y (2001). "Mechanism of 7, 12-Dimethylbenz[a]anthracene-Induced Immunotoxicity: Role of Metabolic Activation at the Target Organ". Jpn J Pharmacol. 86: 302–309. doi:10.1254/jjp.86.302.

[44]. Nakagawa Y, Moore G. (1999). Role of mitochondrial membrane perme¬ability transition in p-hydroxybenzoate ester-induced cytotoxic¬ity in rat hepatocytes. BiochemPharmacol. 1999;58(5):811–6.

[45]. Ou TT, Hsu MJ, Chan KC, Huang CN, Ho HH, Wang CJ. Mulberry extract inhibits oleic acid-induced lipidaccumulation via reduction of lipogenesis and promotion of hepatic lipid clearance. J Sci Food Agric 2011;91:2740-8.

[46]. Peresleni T, Noiri E, Bahou WF, Goligorsky MS. (1996). Antisense oligode¬oxynucleotides to inducible NO synthase rescue epithelial cells from oxidative stress injury. Am J Physiol. 1996;270(6 Pt 2):F971–7.

[47]. Petta S, Craxi A. Hepatocellular carcinoma and non-alcoholic fatty liver disease: from a clinical to a molecular association. Curr Pharmaceut Des 2010;16:741- 52.

[48]. Prabu SM, Muthumani M. Silibinin ameliorates arsenic induced nephrotoxicity by abrogation of oxidative stress, inflammation and apoptosis in rats. MolBiol Rep. 2012;39(12):11201–16.

[49]. Rodriguez M, Estrela J, Ortega A. (2013). Natural polyphenols and apoptosis induction in cancer therapy. J Carcinog Mutagen 2013:6.

[50]. Rohit Arora; Sakshi Bhushan; Rakesh Kumar; Rahul Mannan; Pardeep Kaur; Amrit Pal Singh; Bikram Singh; Adarsh P. Vig; Deepika Sharma; and Saroj Arora (2014). Hepatic Dysfunction Induced by 7, 12-Dimethylbenz (α) anthracene and Its Obviation with Erucin Using Enzymatic and Histological Changes as Indicators. PLoS One. 2014; 9(11): e112614.Published online 2014 Nov 12. doi: 10.1371/journal.pone.0112614 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4229223/

[51]. Russo M, Russo GL, Daglia M, Kasi PD, Ravi S, Nabavi SF, et al. (2016). Understanding genistein in cancer: the “good” and the “bad” effects: a review. Food Chem 2016;196:589-600.

[52]. Saller R, Brignoli R, Melzer J, Meier R (2008). "An updated systematic review with meta-analysis for the clinical evidence of silymarin". Forschende Komplementärmedizin. 15 (1): 9–20. doi:10.1159/000113648. PMID 18334810. Retrieved 2010-12-14.

[53]. Shadnia S, Ashrafivand S, Mostafalou S, Abdollahi M. N-acetyl¬cysteine a Novel Treatment for Acute Human Organophosphate Poisoning. Int J Pharmacology. 2011;7(6).

[54]. Shadnia S, Dasgar M, Taghikhani S, Mohammadirad A, Kho¬rasani R, Abdollahi M. Protective Effects of alpha-Tocopherol and N-

Acetyl-Cysteine on Diazinon-Induced Oxidative Stress and Acetylcholinesterase Inhibition in Rats. ToxicolMech Methods. 2007;17(2):109–15.

[55]. Shah MD, Iqbal M. (2010). Diazinon-induced oxidative stress and renal dysfunction in rats. Food ChemToxicol. 2010;48(12):3345–53.

[56]. Singh, Rana P.; Agarwal, Rajesh (September 2009). "Cosmeceuticals and silibinin". Clinics in Dermatology. 27 (5): 479–484. doi:10.1016/j.clindermatol.2009.05.012. PMC 2767273. PMID 19695480.

[57]. Sirmali M, Solak O, Tezel C, Sirmali R, Ginis Z, Atik D, et al. Com¬parative analysis of the protective effects of caffeic acid pheneth¬yl ester (CAPE) on pulmonary contusion lung oxidative stress and serum copper and zinc levels in experimental rat model. Biol Trace Elem Res. 2013;151(1):50–8.

[58]. Sozmen M, Devrim AK, Tunca R, Bayezit M, Dag S, Essiz D. (2014). Protec¬tive effects of silymarin on fumonisin B(1)-induced hepatotoxic¬ity in mice. J Vet Sci. 2014;15(1):51–60.

[59]. Stagos D, Amoutzias GD, Matakos A, Spyrou A, Tsatsakis AM, Kouretas D. Chemoprevention of liver cancer by plant polyphenols. Food Chem Toxicol 2012;50:2155-70.

[60]. Stagos D, Amoutzias GD, Matakos A, Spyrou A, Tsatsakis AM, Kouretas D. Chemoprevention of liver cancer by plant polyphenols. Food Chem Toxicol 2012;50:2155-70.

[61]. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. Hepatology 2010;51:1820-32.

[62]. Sung YM; He G; Fischer, SM (2005). "Lack of Expression of the EP2 but not EP3 Receptor for Prostaglandin E2 Results in Suppression of Skin Tumor Development". Cancer Res. 65: 9304–9311. doi:10.1158/0008-5472.can-05-1015.

[63]. Teimouri F, Amirkabirian N, Esmaily H, Mohammadirad A, Ali¬ahmadi A, Abdollahi M. (2006). Alteration of hepatic cells glucose me¬tabolism as a non-cholinergic detoxication mechanism in coun¬teracting diazinon-induced oxidative stress. Hum ExpToxicol. 2006; 25(12):697–703.

[64]. Thomas W. Flaig; Daniel L. Gustafson; Lih-Jen Su; Joseph A. Zirrolli; Frances Crighton; Gail S. Harrison; A. Scott Pierson; Rajesh Agarwal; L. Michael Glodé (2007). "A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients". Investigational New Drugs. 25 (2): 139–146. doi:10.1007/s10637-006-9019-2.

[65]. Trappoliere M, Caligiuri A, Schmid M, Bertolani C, Failli P, Vizzutti F, et al. Silybin, a component of sylimarin, exerts anti-inflamma¬tory and anti-fibrogenic effects on human hepatic stellate cells. J Hepatol. 2009;50(6):1102–11.

[66]. Trouillas P, Marsal P, Svobodova A, Vostalova J, Gazak R, Hrbac J, et al. Mechanism of the antioxidant action of silybin and 2,3-dehy¬drosilybin flavonolignans: a joint experimental and theoretical study. J PhysChem A. 2008;112(5):1054–63.

[67]. Tůmová L, Tůma J, Megušar K, Doleža M (2010). "Substituted Pyrazinecarboxamides as Abiotic Elicitors of Flavolignan Production in Silybummarianum (L.) Gaertn Cultures in Vitro". Molecules. 15 (1): 331–340. doi:10.3390/molecules15010331.

[68]. Tutanc M, Arica V, Yilmaz N, Nacar A, Zararsiz I, Basarslan F, et al. Effects of erdosteine on cyclosporin-A-induced nephrotoxicity. Hum ExpToxicol. 2012;31(6):565–73.

[69]. van Wenum E, Jurczakowski R, Litwinienko G. Media effects on the mechanism of antioxidant action of silybin and 2,3-dehydro¬silybin: role of the enol group. J Org Chem. 2013;78(18):9102–12.

[70]. Vitthalrao B. Khyade and Sharad G. Jagtap (2016). Antioxidant activity and phenolic

compounds of mulberry, Morus alba (L) (Variety: Baramatiwali) International Conference on "Plant Research and Resource Management" And 25th APSI Silver Jubilee Meet 2016 at T. C. College Baramati 11, 12 and 13 February, 2016. Pages: 374 – 377.

[71]. Vitthalrao B. Khyade (2016).Impact of Moracin on Hydrogen Peroxide induced oxidative stress in feline fibroblasts.International Academic Journal of Innovative Research. Vol. 3No.10 : 45 - 59 . ISSN 2454-390X. www.iaiest.com

[72]. Vitthalrao B. Khyade (2016).Induction of buccal pouch carcinoma through 7, 12 – dimethylbenz(a) anthracene (DMBA) in Syrian hamster, Mesocricetu sauratus (L) and treatment with the ethanolic extractives of leaves of mulberry, Morus alba (L). International Academic Journal of Innovative Research. Vol. 3 No. 10: 68 - 83 . ISSN 2454-390X. www.iaiest.com

[73]. Vitthalrao B. Khyade ;Vivekanand V. Khyade ; Sunanda V. Khyade and May-Britt Moser (2014). Influence ofMoracin on DMBA-TPA induced skin tumerigenesis in the mouse. International Journal of Bioassays 3 (11): 3510 – 3516. ISSN: 2278-778X. www.ijbio.com

[74]. Vitthalrao B. Khyade and Sadhana D. Deshpande (2015). Chemopreventive Efficacy of Ethanolic Extractives of Leaves of Mulberry, Morus alba (L) ON 7, 12- Dimethylbenz Anthracene (DMBA) induced Buccal Pouch Carcinoma in Syrian Hamster, Mesocricetus auratus (L).International Journal of Recent Scientific Research ,Vol. 6, Issue, 3, pp.3156-3161, March, 2015. www.recentscientific.com

[75]. Vitthalrao B. Khyade and Ujwala D. Lonkar (2013):Effect of Moracin on DMBA – TPA induced cancer in mice, Mus musculus(L). 2013.Annals of Plant Science Vol. 2 No. 10 (2013):412 – 419. http://ebioscholar.com/ojs/index.php/ap/article/view/628/528

[76]. Vitthalrao B. Khyade; Rhidim Mote and Jiwan P. Sarwade (2014). Influence of Moracin on DMBA – TPA induced cancer in the skin of mice, Mus musculus (L). Recent Trends in Zoology (Pages: 113-133). Editor: Dr. R. K. Kasar ; Publisher: Dr. L. S. Matkar (Principal, New Arts, Commerce and Science College, Shevgaon Dist. Ahmednagar – 414502 (M.S.) India. ISBN: 978-93-84916-68-8.

[77]. Vitthalrao B. Khyade; Vivekanand V. Khyade and Sunanda V. Khyade(2013): Use of Moracin in preventing the cancer. Journal Of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT) e-ISSN:2319-2402,p- ISSN: 2319-2399. Volume 4, Issue 5 (May. - Jun. 2013), PP 96-104www.Iosrjournals.Org

[78]. Vitthalrao B. Khyade1 and Aziz Sancer (2016).Treating the 7,12-dimethylbenz(a) anthracene (DMBA) induced buccal pouch carcinoma in Syrian hamster, Mesocricetus auratus(L) with ethanolic extractives of leaves of mulberry, Morus alba (L).World Scientific news 30 (2016): 1-13. www.worldscientificnews.com

[79]. Vitthalrao B.Khyade ;Suryakant M. Mundhe and Shakir Ali Syed (2015). Influence of Ethanolic Extractives of Leaves of Mulberry, Morus alba (L) On 7, 12-Dimethylbenz (A) Anthracene (DMBA) Induced Buccal Pouch Carcinoma in Syrian Hamster, Mesocricetus auratus (L). IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 1 Ver. IV (Jan -Feb. 2015), PP 69-75www.iosrjournals.org

[80]. Vitthalrao Bhimasha Khyade and Avram Hershko (2018). Use of Aqueous Decoction of Leaves of Mulberry, Morus alba (L) in Hyperglycemia-Induced Oxidative Stress in Brown Rat, Rattus norvegicus (L.). International Journal of Agro-Chemistry Vol 4, No 2 (2018).

[81]. Voinovich D, Perissutti B, Grassi M, Passerini N, Bigotto A (2009). "Solid state mechanochemical

activation of Silybummarianum dry extract with betacyclodextrins: Characterization and bioavailability of the coground systems". Journal of Pharmaceutical Sciences. 98 (11): 4119–29. doi:10.1002/jps.21704. PMID 19226635.

[82]. Wu JW, Lin LC, Tsai TH (2009). "Drug-drug interactions of silymarin on the perspective of pharmacokinetics". Journal of Ethnopharmacology. 121 (2): 185–93. doi:10.1016/j.jep.2008.10.036. PMID 19041708. Retrieved 2010-12-14.

[83]. Yang M-Y, Chang Y-C, Chan K-C, Lee Y-J, Wang C-J. Flavonoid-enriched extracts from Nelumbo nucifera leaves inhibits proliferation of breast cancer in vitro and in vivo. Eur J Integr Med 2011;3:e153-63.

[84]. Yang M-Y, Chang Y-C, Chan K-C, Lee Y-J, Wang C-J. (2011). Flavonoid-enriched extracts from Nelumbo nucifera leaves inhibits proliferation of breast cancer in vitro and in vivo. Eur J Integr Med 2011;3:e153-63.

[85]. Yehia MA, El-Banna SG, Okab AB. (2007).Diazinon toxicity affects histo¬physiological and biochemical parameters in rabbits. Exp Toxicol Pathol. 2007;59(3-4):215–25.

[86]. Yilmaz N, Yilmaz M, Altuntas I. (2012). Diazinon-induced brain tox¬icity and protection by vitamins E plus C. ToxicolInd Health. 2012;28(1):51–7.

[87]. ZarichiBaghlani K, Saberi M, RasooliVani J. Protective effects of guanosine on Diazinon induced oxidative stress in neuralgia U373MG cell line. Trauma Mon. 2011;16(3):157–61.

[88]. Zhou S, Lim LY, Chowbay B (2004). "Herbal modulation of P-glycoprotein". Drug Metabolism Reviews. 36 (1): 57–104. doi:10.1081/DMR-120028427. PMID 15072439.

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