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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 12-0-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 include: 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 lepidopteran 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 nphroprotective influence in hyperglycemia-induced oxidative stress in brown rat *Rattus norvegicus* (L) (Vithalrao 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 Shetí 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 norwegicus (L), fifty adult female (12 week-old) Brown rats (Rattus norwegicus 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 Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardonagar 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 Zoology, Shardabai Pawar Mahila Mahavidyalaya, Shardonagar Baramati). The experimental animals were divided into four groups, each with ten individuals. Remaining ten individuals of experimental animals were maintained as reservoir. The individuals of group 1 were served as untreated control group. The individuals of this untreated control group received 0.3 ml corn oil daily 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 intraperitoneal 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 intraperitoneal 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 BioTek 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 Kruskal-Wallis analysis of variance for unpaired comparisons, followed by the Mann Whitney U test. The P < 0.05 was considered significant.

**III. RESULTS AND DISCUSSION**

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 aspartateamino 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>
<thead>
<tr>
<th>Groups</th>
<th>AST, U/L</th>
<th>ALT, U/L</th>
<th>SOD, U/m L</th>
<th>GPx, U/m L</th>
<th>NO, μm/g</th>
<th>MP O, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>108.71 (± 23.604)</td>
<td>55.681 (±13.173)</td>
<td>0.10 (± 0.02)</td>
<td>3.51 (± 0.98)</td>
<td>1.44 (± 0.73)</td>
<td>1.52 (± 1.03)</td>
</tr>
<tr>
<td>DMBA group</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80.71 (± 14.039)</td>
<td>90.906 (± 10.281)</td>
<td>0.09 (± 0.02)</td>
<td>3.74 (± 2.79)</td>
<td>7.20 (± 2.75)</td>
<td>4.78 (± 1.61)</td>
</tr>
<tr>
<td>AMLD group</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>91.40 (± 11.362)</td>
<td>45.328 (± 3.22)</td>
<td>3.72 (± 2.60)</td>
<td>8.02 (± 2.70)</td>
<td>1.27 (± 0.78)</td>
<td>1.21 (± 0.51)</td>
</tr>
<tr>
<td>DMBA + AMLD group</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>106.05 (± 23.573)</td>
<td>54.792 (± 10.089)</td>
<td>0.52 (± 0.34)</td>
<td>7.54 (± 2.45)</td>
<td>2.56 (± 1.36)</td>
<td>0.81 (± 0.33)</td>
</tr>
</tbody>
</table>

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.

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).
Fig. 1: Serum Aspartate Aminotransferase and Alanine Aminotransferase Activities in Norwegian Rat, *Rattus norvegicus* (L) recipient 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 Parameters of Liver in DMBA Induced Hepatotoxicity and Free-Radical Damage in Norwegian Rat, *Rattus norvegicus* (L).

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

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.
The velocity of biochemical reaction catalyzed by the enzymes like asparate amino 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 asparate amino 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 homogenate 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 homogenate 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 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 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 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 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 amount 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 peroxidation 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 synthesizes hypochlorous acid from H₂O₂ 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 phospholipids 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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