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Madhuca longifolia ethanolic seeds extract attenuates chemical carcinogenesis-induced oxidative injury through antioxidation and inhibition of DNA damage to skin and liver cancer in Swiss albino female mice model

[1*]Dibyajyoti Saha and [1]Satish Kumar Sarankar

[1]Faculty of Pharmacy, Mansarovar Global University, Billkisganj, Sehore-466001 (India)
[*]Corresponding Author: djsmgupharmacy@gmail.com

Abstract

Epidemiological and experimental evidence suggested that phytochemicals have potent cancer chemopreventive agent and also active against skin and liver cancer. Exhaustive studies revealed that the *Madhuca longifolia* have more potential as chemopreventive agents due to their lower toxicity. DMBA known as tumor initiator and croton oil is a highly active tumor promoter. The purpose of the research was to explore the chemopreventive activity of *Madhuca longifolia* ethanolic seeds extract in a 7,12 dimethylbenz (a) anthracene (DMBA)-croton oil carcinogenesis model. Five groups were designed for the study and Swiss albino female mice were intraperitoneally injected with vincristine 50mg/kg b.w. at a standardized dose. *Mahua* extract was given orally at an optimum dose of 15 mg/kg b.w. and 30 mg/kg b.w. as per experimental protocol. The parameters LPO, GST, SOD, CAT, GSH have been evaluated. The results obtained showed significant elevation of glutathione-s–transferase, superoxide dismutase, catalase, reduced glutathione and inhibition of lipid peroxidation in skin and liver. The results obtained showed significant reduction of the number of skin papillomas along with elevation of detoxifying enzymes and inhibition of lipid peroxidation in the skin. The results established that the plant seeds have potent skin and liver cancer chemopreventive agent. Further studies need to be done to understand the mechanism of action.

Key words : Carcinogenesis, Chemical, Chemoprevention, Liver Cancer, *Madhuca longifolia*, Skin Cancer.

Skin cancer is one of the most common cancer affecting humans worldwide and its incidence is rapidly increasing. The study of skin and liver carcinogenesis is of major interest for both scientific research & clinical practice and the use of in vivo systems

may facilitate the investigation of early alterations in the skin and liver. When it comes to primary liver cancer, malignant (cancerous) liver tumours either originate there or spread from other disease sites throughout the body (metastatic liver cancer). Metastatic cancerous tumours make up the vast bulk of liver tumours. Cancer chemoprevention approaches are aimed at preventing, delaying or suppressing tumor incidence using synthetic or natural bioactive agents[8]. Cancer chemoprevention is the use of natural and synthetic agents to suppress, prevent or delay tumorigenesis by blocking the initiation stage of carcinogenesis, or by curtailing the promotion stage wherein the initiated cells proliferate to give rise to a tumor[7]. Phytochemicals are chemicals which are found in plants also include indoles, lignans, phytoestrogens, stanols, saponins, terpenes, flavonoids, carotenoids, anthocyanidins and phenolic acids[2]. The evidence suggested that prolonged oxidative and nitrosative stress can result in cell injury and that they play roles in various stages of carcinogenesis[16]. Tumor cells present de-regulated cell proliferation and differentiation, and acquire autonomous and unlimited growth properties together with resistance to apoptosis[17]. Their growth also stimulated by reactive oxygen species. Free radicals, generally composed of reactive oxygen species (ROS) and reactive nitrogen species (RNS), are generated in the body by various endogenous and exogenous systems[22]. The overproduction of free radicals also known to cause several chronic diseases including cancer. Xenobiotics explored the chemical substances that are foreign to animal life and thus includes such examples as plant constituents, drugs, pesticides, cosmetics, flavorings, fragrances, food additives, industrial chemicals and environmental pollutants[9]. The role of metabolic activation in carcinogenesis and the importance of DNA damage and mutation have led to additional avenues of research regarding mechanisms of carcinogenesis and influences on the carcinogenic process. The first of these is DNA repair[6]. Xenobiotics also considered as chemical substances from natural or synthetic sources found within an organism that are not naturally produced by the organism or expected to be present[18]. Oxidative stress (OS) expressed as a state of excess ROS production and/or the reduction in savaging antioxidants, which results in pathophysiological changes similar to the general adaption syndrome of cellular stressors[21]. Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or addition of an oxygen radical, resulting in the oxidative damage of polyunsaturated fatty acids (PUFA)[10]. GST proteins are crucial antioxidant enzymes that regulate stress-induced signaling pathways[15]. Glutathione (GSH) plays an important role in a multitude of cellular processes, including cell differentiation, proliferation, and apoptosis, and disturbances in GSH homeostasis are involved in the etiology and progression of cancer. Catalase also plays an important role in cancer[13]. Antioxidants are secondary constituents or metabolites found naturally in the plants and they produce array of antioxidant compounds that includes carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols to prevent oxidation of the susceptible substrate[5]. *Madhuca longifolia* (J. Koenig Ex L.) J. F. Macbr. (Family: Sapotaceae) have pharmaceutical, nutraceutical, ethno medicinal and ethno pharmacological value. The plant have anti-inflammatory, antipyretic, anti-hyperglycemic,

antifertility, antiulcer, antimicrobial, antioxidant, cardio protective, anti-carcinogenic, immuno-modulant, anti-rheumatic, oxytocic, anti-estrogenic, antiepileptic, demulcents and also other useful pharmacological activities. In this experiment, two chemical carcinogens were used by inducing skin cancers namely 7,12-Dimethylbenz[a]anthracene (tumor initiator) and promoted by croton oil (phorbol ester). Chronic inflammation, oxidative stress and DNA damage were caused by DMBA whereas croton oil induced cellular damage by generating free radicals. As best of knowledge, no cancer chemoprevention work of this plant seeds was previously reported. The aim of research was to find out the chemo preventive efficacy of *Madhuca longifolia* seeds to prevent skin and liver cancer in the model of Swiss albino female mice.

Chemicals :

DMBA, croton oil, ethylene diamine tetra acetic acid (EDTA), pyrogallol, thiobarbituric acid (TBA), bovine serum albumin (BSA), vincristine, 1-chloro-2, 4-dinitrobenzene (CDNB), sodium dodecyl sulphate (SDS), reduced glutathione (GSH), Hydrogen peroxide 30% etc. All the reagents and chemicals were of analytical grade.

Animals :

Adult (five to six weeks) Swiss albino female mice (20 gm to 24 gm), were taken for this study and maintained at twelve hours light/ dark cycle, humidity (55% to 65%), temperature (21ºC to 25ºC). Six mice per cage were housed in wire-mesh cages. Standard food pellets diet (rat/mice feed) and drinking water was provided ad libitum. The animals in each group had their backs shaved two days prior the experiment started.

Preparation of drug :

Ethanolic extract of *Madhuca longifolia* seeds (15mg/30 mg) was dissolved in 200 µl phosphate buffer saline. Each day of the experiment, just before the treatment, it was prepared.

Preparation of carcinogenesis :

DMBA preparation: 100 mg DMBA were taken in a measuring tube using micropipette. Thereafter 9 ml 900 µl acetone were given in a tube and the total volume was 10 ml. The preparation were given tropically and the amount was 100 µl per mouse. So, the DMBA were dissolved of 100 µg / 100 µl per acetone concentration.

Croton oil preparation: 100 µl croton oil were taken in a measuring tube using micropipette. Thereafter 9 ml 900 µl acetone was given in a tube and the total volume was 10 ml. The preparation was given tropically and the amount was 100 µl per mouse. So, the dilution of the croton oil in acetone to give a solution of 1% w/v.

The carcinogenesis was prepared each day of the experiment, just before the treatment.

Experimental design:

Vehicle control group (Group-I): Animals received PBS orally (200 µl/mouse) and topically acetone (100 µ l/mouse) over the skin shaved area for 12 weeks.

Carcinogen control group (Group-II): Animals received two topical applications of DMBA at an interval of 72 h, at a dose of 50mg/kg body weight in acetone (100 µl/ mouse), followed by croton oil (1% w/v) in acetone (100 µl/mouse), twice a week for 9 weeks starting from day 8 of first DMBA application.

Positive control group (Group III): Animals received the same treatment as same as group II and also administered intraperitoneally with 50 mg/kg standard drug of Vincristine from the day (on day 8th after the 1st DMBA application) of croton oil treatment.

Lower dose test group (Group-IV): Animals received the same treatment as for group II and also received the Mahua Extract (ME) at a dose of 15 mg/kg body weight/day orally from the day (on day 8th after the 1st DMBA application) of croton oil treatment.

Higher dose test group (Group-V): Animals received the same treatment as for group II and also received the Mahua Extract (ME) at a dose of 30 mg/kg body weight/day orally from the day (on day 8th after the 1st DMBA application) of croton oil treatment.

After twelve weeks of the first application of DMBA, the animal groups I, II, III, IV, and V were sacrificed according to IAEC standards[19,20].

Detection of Papilloma's :

The experimental animals were carefully examined weekly upto 12th week for counting and recording the incidence of papilloma and the number of papilloma per papilloma bearing mouse. Skin papillomas with a diameter greater than 1mm that persists for at least two consecutive observations were used for counting. The papillomas, which regressed after one observation, were not considered for counting. Two different experts who were not concerned with information regarding the experimental groups performed the measurement of papillomas.

Estimation of Antioxidant and oxidative enzymatic parameters :

Assay of lipid per oxidation (LPO): Lipid peroxidation was estimated in tissue microsomal fraction. The level of lipid peroxides formed was measured using thiobarbituric acid and expressed as nano mole of thiobarbituric acid reactive substances (TBARS) formed per mg of protein using extinction coefficient[11] of 1.56×105 $M^{-1}cm^{-1}$.

Assay of Glutathione - S - Transferase (GST): Glutathione -S-Transferase (GST) activity was measured in the tissue cytosol. The enzyme activity was determined from the increase in absorbance at 340nm with 1-chloro-2-4-dinitrobenzene (CDNB) as the substrate and specific activity of the enzyme expressed as formation of 1-chloro- 2-4-dinitrobenzene (CDNB)-GSH conjugate per minute per mg of protein[1].

Assay of Superoxide Dismutase (SOD): SOD activity in tissue cytosol was assayed and the partial extraction and purification of SOD was done[3]. Superoxide dismutase (SOD) activity was determined by quantification of Pyrogallol auto oxidation inhibition and expressed as unit/mg of protein.

One unit of enzyme activity is defined as the amount of enzyme necessary for inhibiting the reaction by 50%. Auto oxidation of Pyrogallol in Tris-HCL buffer (50 mM, pH 7.5) is measured by increase in absorbance at 420 nm.

Assay of Catalase (CAT): Activity of catalase (CAT) in tissue cytosol was determined spectrophotometrically at 250nm and expressed as units per milligram of protein where the unit is the amount of enzyme that liberates half the peroxide oxygen from H_2O_2 in 100 seconds at 25°C. [12].

Assay of reduced gluthathione (GSH): The activity of GSH was determined as nanomoles per milligram of protein in the cytosol[12].

Statistical analysis :

Each experimental data was presented as MEAN ± SEM. All of the groups were compared and analyzed using a one-way ANOVA and then Dunnett's multiple comparison tests were performed. The values were statistically significant if the p-value[4,14] was less than 0.05.

Effect of ME on the development of papillomas :

The incidence of papillomas was observed. The results were annexed (Table-1).

Table-1. Effect of ME on the development of papillomas

Groups	Treatment	Tumor incidence (%)	Total No. of Tumors
I	Vehicle control group	0	0
II	Carcinogen control group	100	6/6
III	Positive control group	66.66	4/6
IV	Lower dose test group	50	3/6
V	Higher dose test group	33	2/6

After the completion of the 12 weeks experiment, the tumor incidence of the DMBA-croton oil-treated mice (Gr. II) was found 100%. The tumor incidence of positive control group (Group III) treated mice was found 66.66%. Lower dose mice group (Gr. IV) received ME treatment at a dose of 15 mg/kg body weight was found 50% tumor incidence, whereas animals of higher dose mice group (Gr. V) receiving the drug at a dose of 30 mg/kg body weight was found 33% tumor incidence. Animals of Gr. I showed no incidence of papillomas. (Figure 1).

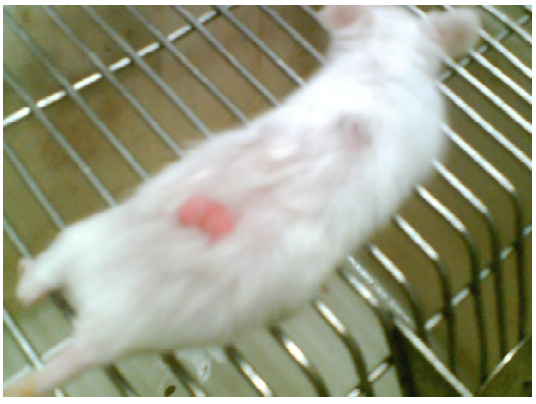

Figure 1. Development of papillomas

Estimation of antioxidant and oxidative enzymatic parameters (Skin):

Lipid peroxidation (LPO): The significant increase was shown of lipid per oxidation in carcinogen control group II (161.5 ± 3.12%) compared to the vehicle control group I (Figure 2).

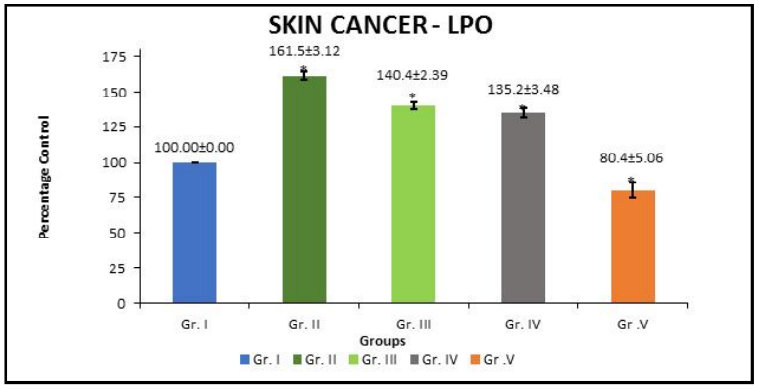

Figure 2. Skin Cancer-LPO

The positive control group III (140.4 ± 2.39%) showed inhibition of lipid peroxidation. However, simultaneous treatment of group IV (135.2 ± 3.48%) showed better result than group III. The highest inhibition of lipid peroxidation showed by higher dose test group V (80.4 ± 5.06%) than group III & IV after 12 weeks treatment.

Glutathione-S-transferase (GST): The significant decrease was shown of glutathione-S-Transferase (GST) in carcinogen control group II (48.65 ± 1.25%) compared to the vehicle control group I (Figure 3).

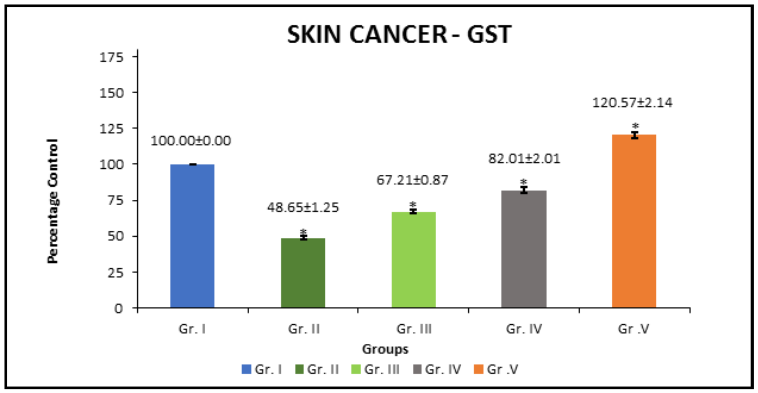

Figure 3. Skin Cancer-GST

The positive control group III (67.21 ± 0.87%) showed significant increase of GST activity. However, simultaneous treatment of group IV (82.01 ± 2.01%) showed better result

than group III. The highest increase of GST showed by higher dose test group V (120.57 ± 2.14%) than group III & IV after 12 weeks treatment.

Reduced gluthathione (GSH): The significant decrease was shown of reduced gluthathione (GSH) in carcinogen control group II (48.08 ± 1.70%) compared to the vehicle control group I (Figure 4).

The positive control group III (62.01 ± 2.89%) showed significant increase of GSH activity. However, simultaneous treatment of group IV (71.21 ± 3.24%) showed better result than group III. The highest increase of GSH showed by higher dose test group V (125.32 ± 2.58%) than group III & IV after 12 weeks treatment.

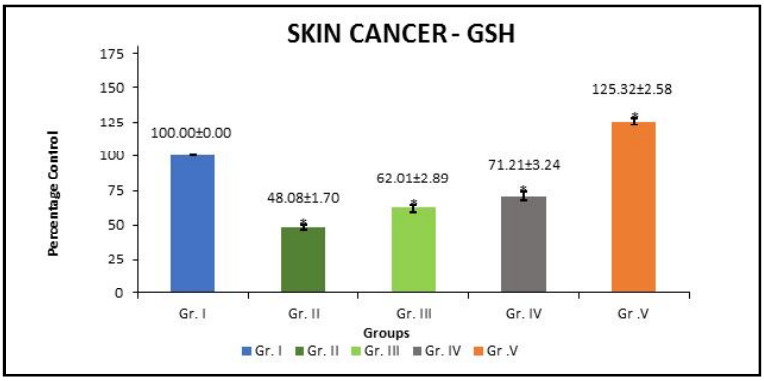

Figure 4. Skin Cancer-GSH

Superoxide dismutase (SOD): The significant decrease was shown of superoxide dismutase (SOD) in carcinogen control group II (38.46 ± 0.55%) compared to the vehicle control group I (Figure 5).

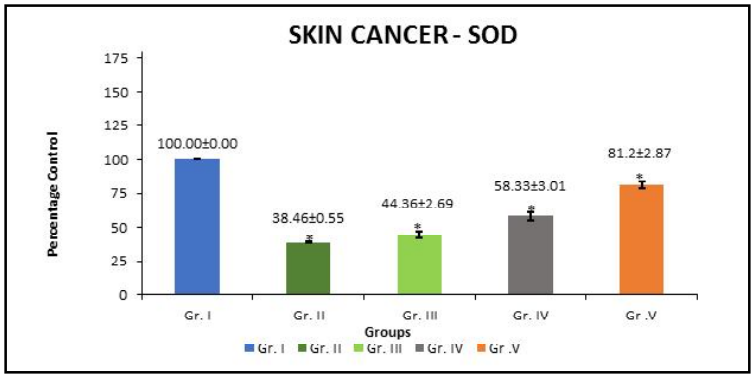

Figure 5. Skin Cancer-SOD

The positive control group III (46.36 ± 2.69%) showed significant increase of SOD

activity. However, simultaneous treatment of group IV (58.33 ± 3.01%) showed better result than group III. The highest increase of SOD showed by higher dose test group V (81.2 ± 2.87%) than group III & IV after 12 weeks treatment.

Catalase (CAT): The significant decrease was shown of catalase (CAT) in carcinogen control group II (25.15 ± 2.17%) compared to the vehicle control group I (Figure 6).

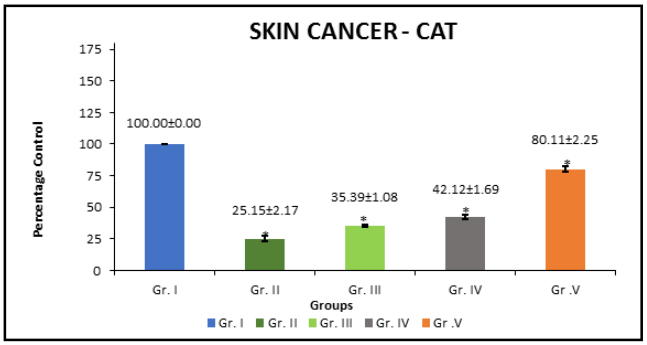

Figure 6. Skin Cancer-CAT

The positive control group III (35.39 ± 1.08%) showed significant increase of CAT activity. However, simultaneous treatment of group IV (42.12 ± 1.69%) showed better result than group III. The highest increase of CAT showed by higher dose test group V (80.11 ± 2.25%) than group III & IV after 12 weeks treatment.

Estimation of antioxidant and oxidative enzymatic parameters (Liver):

Lipid per oxidation (LPO): The significant increase was shown of lipid per oxidation in carcinogen control group II (162.65 ± 2.93%) compared to the vehicle control group I. The positive control group III (134.6 ± 3.42%) showed inhibition of lipid peroxidation. However, simultaneous treatment of group IV (101.5 ± 1.07%) showed better result than group III. The highest inhibition of lipid peroxidation showed by higher dose test group V (66.9 ± 2.33%) than group III & IV after 12 weeks treatment (Figure 7).

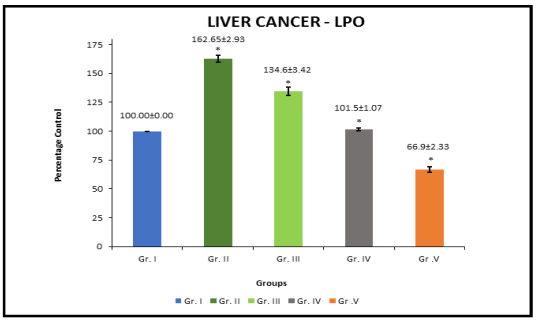

Figure 7. Liver Cancer-LPO

Glutathione - S - Transferase (GST): The significant decrease was shown of glutathione-S-Transferase (GST) in carcinogen control group II (51.13 ± 1.27%) compared to the vehicle control group I. The positive control group III (63.24 ± 0.78%) showed significant increase of GST activity. However, simultaneous treatment of group IV (90.21 ± 3.22%) showed better result than group III. The highest increase of GST showed by higher dose test group V (125.53 ± 2.67%) than group III & IV after 12 weeks treatment (Figure 8).

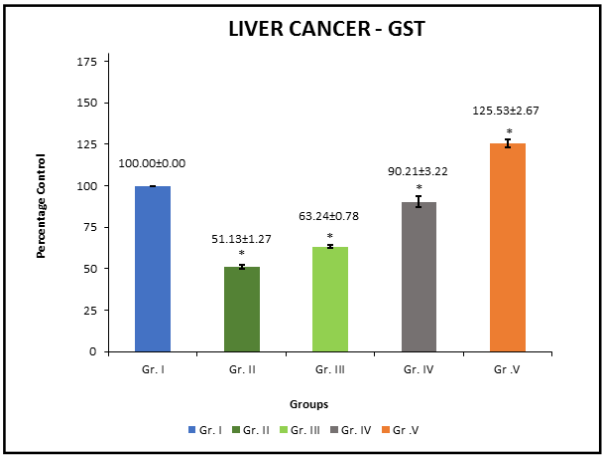

Figure 8. Liver Cancer-GST

Reduced Gluthathione (GSH): The significant decrease was shown of reduced Gluthathione (GSH) in carcinogen control group II (46.05 ± 1.68%) compared to the vehicle control group I. The positive control group III (62.85 ± 2.07%) showed significant increase of GSH activity. However, simultaneous treatment of group IV (80.7 ± 2.83%) showed better result than group III. The highest increase of GSH showed by higher dose test group V (137.58 ± 3.01%) than group III & IV after 12 weeks treatment (Figure 9).

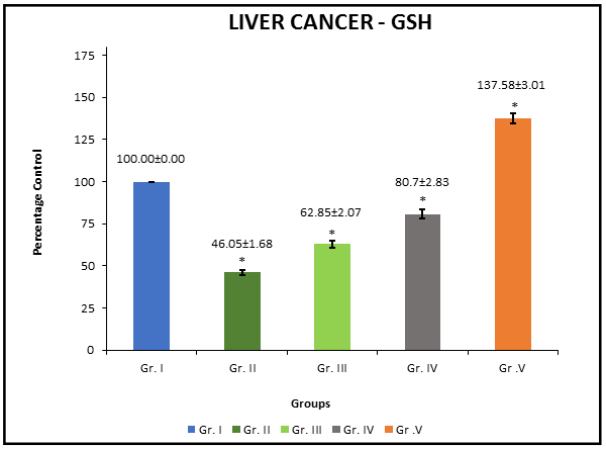

Figure 9. Liver Cancer-GSH

Superoxide Dismutase (SOD): The significant decrease was shown of superoxide dismutase (SOD) in carcinogen control group II (35.78 ± 0.29%) compared to the vehicle control group I. The positive control group III (45.81 ± 2.21%) showed significant increase of SOD activity. However, simultaneous treatment of group IV (62.06 ± 3.01%) showed better result than group III. The highest increase of SOD showed by higher dose test group V (86.05 ± 4.21%) than group III & IV after 12 weeks treatment (Figure 10).

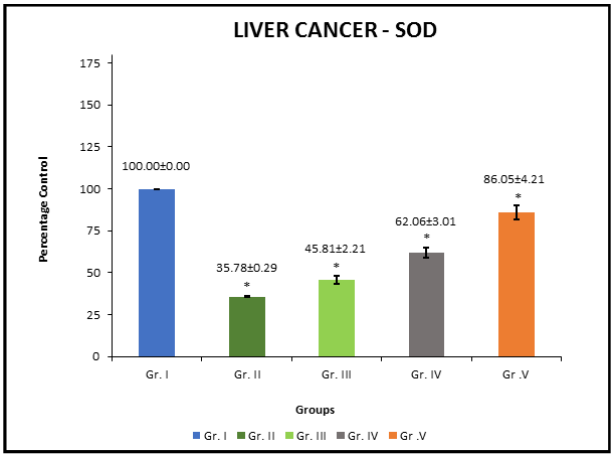

Figure 10. Liver Cancer-SOD

Catalase (CAT): The significant decrease was shown of catalase (CAT) in carcinogen control group II (24.91 ± 2.22%) compared to the vehicle control group I. The positive control group III (41.41 ± 1.65%) showed significant increase of CAT activity. However, simultaneous treatment of group IV (61.21 ± 2.03%) showed better result than group III. The highest increase of CAT showed by higher dose test group V (80.97 ± 3.07%) than group III & IV after 12 weeks treatment (Figure 11).

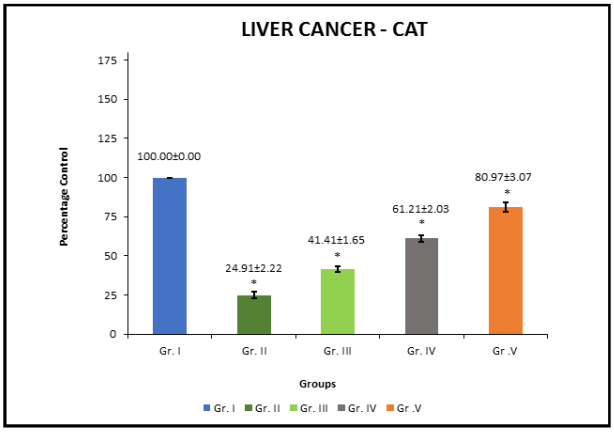

Figure 11. Liver Cancer-CAT

Skin and liver cancer is the most common cancer in the national and international scenario. In the present study, significant DNA damage was observed in carcinogen control group treated animals as compared to the vehicle control group. The number of papillomas per papilloma bearing mouse in the drug treated group were also significantly less compared to the carcinogen control group. Mahua seeds can effectively suppress the proliferation of the cancer cells in this model. However, the DNA damage was reduced significantly when the animals were treated with the positive control and ME groups as compared with the carcinogen control mice. Based on animal protocol, lipid peroxidation was significantly higher in the carcinogen control group II compared to the vehicle control group I for skin and liver cancer and the group III, IV and V were significantly reduced. The GST, GSH, SOD, CAT were significantly lower in the carcinogen control group II compared to the vehicle control group I whereas the group III, IV and V were significantly increased. In conclusion, the results showed that Mahua seed extracts showed chemopreventive effect against chemical carcinogenesis and prevent DNA damage through modulating the antioxidant and detoxifying enzymes. The present research results showed that ethanolic extract of *Mahua longifolia* seeds can actively inhibit the growth of skin and liver cancer cells and also emphasize the potent anticancer activity *in vivo*.

Our research concluded that the seeds of *Mahua longifolia* have potential anti-carcinogenic activity against skin and liver cancer and may act as a potent chemopreventive agent. Therefore, the plant seed extract can be utilized as a chemopreventive or therapeutic agent in skin and liver cancer. Hence, further research to be carried out to find the biologically active compounds present in the seeds of *Mahua longifolia* medicinal plant for anti-skin and anti-liver cancer activity.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Consideration :

This study was performed in accordance with the guidelines for the care and purpose of laboratory animals (No. Ph.D./MGU/Regn. /PHR/ 21/12). All the experiments were carried out in accordance with the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Authors Contribution :

DS: Concept and design of the work, Data collection, Data analysis and Interpretation, Drafting of the article.

SKS: Concept and design of the work, Drafting of the article.

References :

1. Ali, H. and S. Dixit, (2015) *Jour. of Biomed. Res., 29*(2), 139-144. https://doi.org/10.7555/jbr.29.20130025.
2. Alyoussef, A. (2023) *Cureus., 15*(5): e38596. https://doi.org/10.7759/cureus.38596
3. Becker, A.L. and A.K. Indra, (2023) *Cancers., 15:* 3038, 1-18. https://doi.org/10.3390/cancers15113038
4. Bhuyan, D, N. Dua, and T. Kothari, (2016) *Open Journal of Psycho. & Allied Sci, 7*(1): 87. https://doi.org/10.5958/2394-2061.2015.00022.1
5. Buja, A., M. Rivera, G. Girardi, A. Vecchiato, V. Rebba, and E. Pizzo, (2020) *J Med Screen., 27*(3): 157-67.
6. Cadet, J., and T. Douki, (2018) Formation of UV-Induced DNA *Photochem Photobiol Sci., 17*(12): 1816-1841. https://doi.org/10.1039/C7PP00395A
7. Dakrory, A.I., S.R. Fahmy, A.M. Soliman, A.S. Mohamed, and S.A. Amer, (2015) *Biomed Res Int.,* 563652. https://doi.org/10.1155/2015/563652
8. Das, R.K., S. Das, and S. Bhattacharya, (2004) *J Environ Pathol Toxicol Oncol., 23*(4): 287-96.

https://doi.org/10.1615/jenvpathtoxoncol.v23.i4.50

9. Elmets, C.A., and Y. Nabiha, (2020) *Biomed Hub., 5*(2): 958-973, https://doi.org/10.1159/000508295.
10. Gordon, L.G., C. Olsen, D.C. Whiteman,T.M. Elliott, M. Janda, and A.C. Green, (2020) *BMJ Open., 10*(2): 1-13.
11. Kaushik, M.L., M. Singh, D. Rani and R. Singh, (2023) *Int. Journ. Pharm. Scs. and Res., 14* (4): 1754-1761.
12. Kwei, K.A., J.S. Finch, E.J. Thompson, and G.T. Bowden, (2004) *Neoplasia., 6*(5): 440-448. https://doi.org/10.1593/neo.04127
13. Le Bras, A. (2020) *Lab Anim., 49:* 20. https://doi.org/10.1038/s41684-019-0447-y
14. Morgan, D. (2018). *Pharmaceutical Statistics, 17*(4): 298-300. https://doi.org/10.1002/pst.1876
15. Ray, S., N. Murmu, J. Adhikari, S. Bhattacharyya, S. Adhikari, and S. Banerjee, (2014). *Jour. of Env. Path, Tox. & Oncology. , 33*(4): 295-314, 2014. https://doi.org/10.1615/JEnviron Pathol ToxicolOncol.2014011354
16. Shea, L.K., N.S. Akhave, L.A. Sutton, L.A. Compton, C. York, S.M. Ramakrishnan, C.A. Miller, L.D. Wartman, and D.Y. Chen, (2023). *J Invest Dermatol., 143*(2): 232-241.e6. https://doi.org/10.1016/j.jid.2022.08.037
17. Surien, O., S.F. Masre, D.F. Basri, and A.R. Ghazali, (2022). *Biomedicines. 10*(11): 2743. https://doi.org/10.3390/biomedicines10112743
18. Tahir, M., A. Naeem, H. Malik, J. Tanveer, R.A. Naqvi, and S-W. Lee, (2023) *Cancers., 15*(7): 2179. https://doi.org/10.3390/cancers15072179
19. Thorat, R. (2022) *Laboratory Animals., 56*(4): 317-318. https://doi.org/10.1177/00236772221086392
20. Yadav, S. (2020) *Asian Law & Public Pol. Rev., 5:* 114-122.
21. Yusuf, N., T. Haarmann-Stemmann, and M. Nakamura (2022) *Fron in oncology. 12:* 924225. https://doi.org/10.3389/fonc.2022.924225
22. Zeinali, M., S. Abbaspour-Ravasjani, T. Soltanfam, A.C. Paiva-Santos, H. Babaei, F. Veiga, and H. Hamishehkar, (2021) *Life Sci., 283:* 119759. https://doi.org/10.1016/j.lfs.2021.119759