Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Modulator Effect of Mangiferin on Biochemical Characterization in 7,12-Dimethylbenz[a]Anthracene-Induced Oral Cancer in Experimental Hamsters

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Submitted: 19-Jul-2019 Revised: 27-Aug-2019 Accepted: 17-Feb-2020 Published: 11-Nov-2021

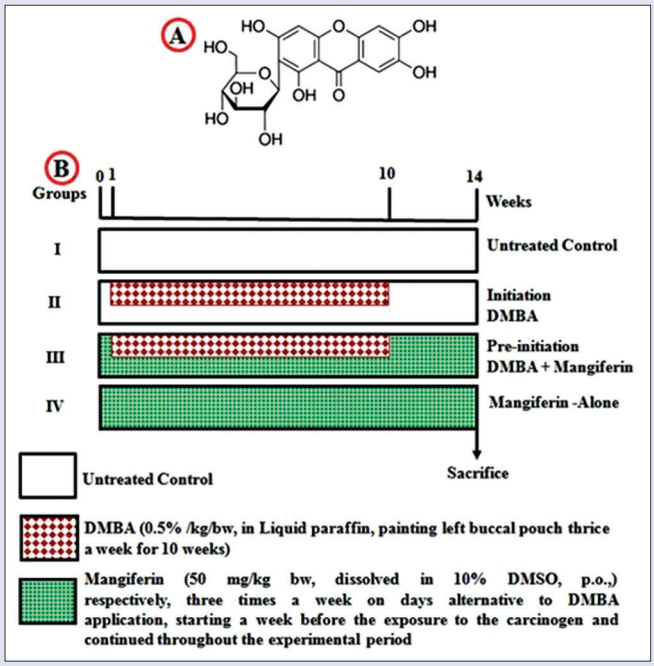
ABSTRACT

Background: Newly, chemopreventive technique might be a hopeful advancement in developing countries for treating cancers with the aid of toxic less natural-based constituents. Malignancy urges to augment effectual chemopreventive agents that are looking forward to suppressing the tumors which may be stimulated by chewing and smoking of tobacco and over alcohol consumption related to the high prevalence of human oral cancer (OC) patients. **Materials and Methods:** In the present research, we examined to assess antioxidants, lipid peroxidation (LPO), and detoxification enzymes levels of anticancer activity of mangiferin on 0.5% 7,12-dimethylbenz[a]anthracene (DMBA) provoked hamster cheek pouch carcinoma. OC on hamster buccal pouch (HBP) was incited by DMBA treatment for thrice per week for over 14 weeks. **Results:** About 100% well-defined OC establishment with body weight (b.wt), tumor burden, antioxidant, LPO and liver marker enzymes, and also histological changes were observed on DMBA-challenged buccal pouch carcinoma (BPC) in hamsters. Orally treated mangiferin at an effective dosage of 50 mg/kg b.wt, to DMBA painted hamsters were significantly averted the b.wt, succession of tumor, the biochemical as well as histopathological changes. **Conclusion:** The findings of this work clearly suggest that the anti-carcinoma effect of mangiferin possess the modulatory effects on potent antioxidant, anti-LPO, and detoxification agents to expel the metabolites of malignant cells, on DMBA-provoked BPC in hamsters.

Key words: Antioxidant, chemopreventive, detoxifying agent, dimethylbenz[a]anthracene, lipid peroxidation, mangiferin, oral cancer

SUMMARY

- Mangiferin is an assured pharmacological activity such as antioxidant, anticancer, antidiabetic, antioxidative, immunomodulatory, and hepatoprotective effects in various diseases
- Mangiferin exerts its prevented 7,12-dimethylbenz[a]anthracene-induced oral cancer in the hamster cheek pouch via its anti-cell proliferative anti-lipid peroxidation and antioxidant possible and also modulating the prominence of Phase-I and II hepatoprotective mediators.



Abbreviations used: OC: Human oral cancer; LPO: Lipid peroxidation; DMBA: 7,12-dimethylbenz[a]anthracene; TB: Tumorburden; BPC: Buccal pouch carcinogenesis.

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DOI: 10.4103/pm.pm_313_19

Access this article online
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INTRODUCTION

Mouth cancer, a class of head-and-neck carcinoma, is a major one and most common malignant condition on the global population.[1] Oral squamous cell carcinoma (OSCC), by means of changeover from an epithelial to a mesenchymal phenotype are hallmark in the competence of self-regulating tumor cell growth toward migrate, invade, and metastasize.[2] Therefore, 80% of patients have suffered from oral cancer (OC) through widespread exposures for the stimulation and progression of mouth carcinoma such as excess alcohol consumption,

Cite this article as: Liu M, Wen C, Pan S. Modulator effect of mangiferin on biochemical characterization in 7,12-dimethylbenz[a] anthracene-induced oral cancer in experimental hamsters. Phcog Mag 2021;17:605-14.

tobacco smoking, and chewing. Although widespread development over finding and progression on behavior approach, OSCC at rest together drastically augmented death rate and morbidity as well.[3] Furthermore, to primary anticipation via the removal of tobacco expenditure, chemoprevention of OC has gained momentum in current years.

Chemoprevention practice might be an endowed improvement in developing countries of the world for the treatment of carcinogenesis. A chemoprevention, an emerging technique was covenants with the suppression of malignant growth with the aid of natural herbal-based compounds.[4] The carcinogenesis suppression capacity of herbal founded phytoconstituents are necessitated to explore by adopting the well established *in vivo* system as well as evidence suggests that the majority used animal models in OC investigate are the male golden Syrian hamster cheek pouch (HCP) with 7,12-dimethylbenz[a] anthracene (DMBA).[5,6] DMBA is polycyclic aromatic hydrocarbons contain large class of compounds presenting powerful carcinogen, co-carcinogen, and tumor promoters. The cause of these carbon molecules are usually noticeable via degradation/loss of nuclear contents, which when nor repaired and in stagnant mutations of trouble genes originating their multiplication.[7] Hence, in our research, we adopted a DMBA provoked mouth cancer model on hamsters which involve a typical administration of DMBA in thrice per week for over 14 weeks regimen. OC induced by DMBA in the HCP protocols in this model induces premalignant changes and carcinomas reiterate many of features that look a lot like human OSCC, which is give out as an outstanding target organ for chemointervention for the purpose that of easy convenience and follow-up of lesions.[8]

Mangiferin is a natural polyphenol derived from edible herbals and it was distributed on numerous components of *Mangifera indica* as well as the fruit peelings, stalk, leaf, bark, and kernel of the mango tree. Mangiferin demonstrated various cellular and experimental models experimentally evaluated its effective function on different malignancies and other ailments.[9] Mangiferin acquired an assured pharmacological activity such as antioxidant, anticancer, antidiabetic, antioxidative, immunomodulatory, and hepatoprotective effects in various diseases.[10] There is good proof for the chemopreventive activity of mangiferin in rodent models, in which it has been shown to inhibits tumor growth in mouse metastatic melanoma,[11] hepatic tumor growth in murine,[12] lung carcinogenesis in Swiss albino mice,[13] lung injury in mice,[14] and dermatitis in a mice.[15] It also suppressed human breast cancer cells.[16] In our research work, we planned to explore the relative antioxidant capacity of mangiferin (50 mg/kg b.w.), an effective dose for inhibitory efficacy on DMBA-challenged HBPC. The different parameters were adopted to investigate the chemo-preventive efficacy of mangiferin against DMBA-provoked mouth cancer in the hamster model.

MATERIALS AND METHODS

Chemicals and reagents

Mangiferin [Figure 1a] DMBA and liquid paraffin (LP) were purchased by Sigma Aldrich Chemicals Pvt. Ltd., (US). The entire erstwhile chemicals were adopted of diagnostic status, obtained from Hi-Media Lab Pvt. Ltd., (US).

Animals

8–10 weeks aged Hamsters (*Mesocricetus auratus*), weighing about 80–120 g was maintained in the central animal house The First Affiliated Hospital of Zhengzhou University. They were residence five propylene cages; all cage limited 6 hamsters were maintained separately and had access to pelleted diet with H_2O *ad libitum*. Hamsters were housed in the guarded situation with 27°C ± 2°C temperature and 55% ±5% moistens with a 12 h light/dark series.

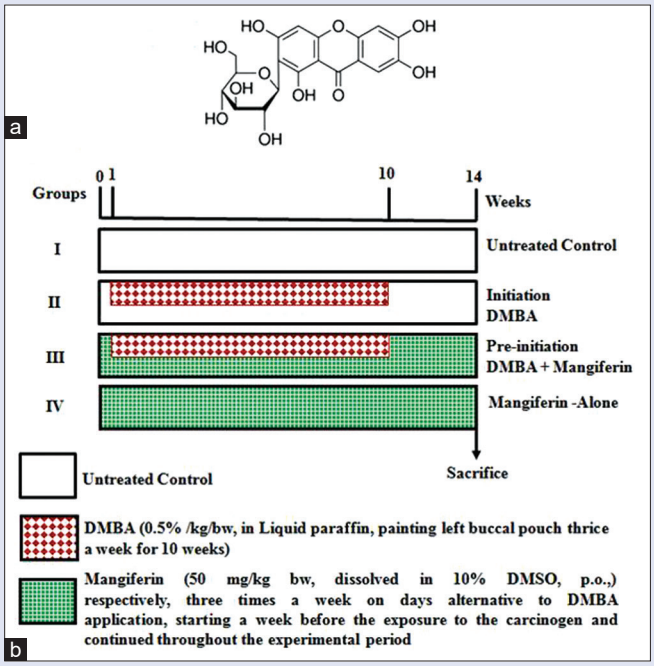

Figure 1: (a) Structure of mangiferin. (b) Experimental protocol for effective dose study

Experimental design

The whole set of animals (24 hamsters) were alienated into four groups with six hamsters in each. Figure 1b shows in the experimental design for effective dose study. Group I hamsters were supplied as normal control animals. Group II and III hamsters were treated with 0.5% DMBA in LP three times for a week to 14 weeks regimen in their gone hamster buccal pouch (HBP) using a number four brush. Group III hamsters received in orally pretreatment with 50 mg/kg Bodyweight (b.wt) of mangiferin, suspended on corn oil, starting 1 week before the disclosure of carcinogen and sustained in alternate periods to DMBA challenge at the end of 14 weeks. Oral treatment of 50 mg/kg b.wt/day of mangiferin was given to Group IV hamsters in unaccompanied during the study regimen. The experimentations were finished on the 10th week and every hamster was killed after anaesthetization via displacement of the cervical bone.

B.wt of studied hamsters was measured using subtraction of earlier and final b.wt. Collected hamster's plasma was utilized for biochemical studies and liver and cheek pouch tissues were exploited for histopathological examine, for this collected cheek pouch was immediately trenched on formalin solution and the processed tissues were implanted in paraffin wax, pieces were sliced with the aid of microtome and colored by hematoxylin and eosin were conducted on untreated control and treated hamsters.

Tumor assessment

Total tumors on every HCP were inspected macroscopically, then hamsters were euthanized and the width of every tumor was calculated with the aid of Vernier meter. The volumes of tumors were determined by applying the formula of $V = 4/3\pi$ (D1/2) (D2/2) (D3/2) where D_1, D_2, and D_3 mean the three widths (mm^3) of tumor. The tumor burden (TB) was proposed via multiplication of the volume of tumor and tumor numbers for each HBP.

Biochemical analysis

Sample collection

After anesthetic hamsters killed, the blood was gathered on heparin painted containers and utilized to separate the plasma by spinning the tube at 3000 rpm for 20 min. Liver and HBP tissues were detached and cleaned by icy buffered saline pulverized to become homogenize and then, the homogenized suspensions were utilized for biochemical investigations.

Total protein substances were calculated approximately by adopting the Lowry *et al.* method.[17] For confirming the generation of Thiobarbituric acid reactive substances (TBARS), lipid hydroperoxide (LOOH) and conjugated (CD), on plasma and oral mucosa the lipid peroxidation (LPO) was determined by applying the method of Ohkawa *et al.*,[18] Jiang *et al.*[19] and Rao and Recknagel,[20] in that order. Enzymatic functions of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) content on the plasma and buccal mucosa was calculated by the procedure of Kakkar *et al.*,[21] Rotruck *et al.*[22] and Sinha,[23] in that order. GSH and Vit-E levels on plasma and buccal mucosa were resolute with the procedure of Beutler and Kelly,[24] Desai,[25] and Palan *et al.*,[26] correspondingly.

Liver marker enzymes such as cyt-p450 and cyt-b5, DT-diaphorase (DTD), glutathione S-transferase (GST), glutathione reductase (GR), glutathione (GSH) and oxidized glutathione (GSSG) levels on liver and oral mucosal tissues were established by adopting the procedure of Omura and Sato,[27] Lind *et al.*,[28] Habig *et al.*,[29] Carlberg and Mannervik,[30] Anderson,[31] Tietze,[32] and Ernster,[33] accordingly. Protein detection was done with the aid of Bradford protein detection colorimetric kits (Bio-Rad, CA).

Table 1. The initial and final body weight of control and experimental hamsters.

Groups	Treatment	Initial body weight	Final body weight
1	Control	112.65±8.58	143.03±10.89[a]
2	DMBA	114.04±8.68	93.15±7.09[b]
4	DMBA + Mangiferin (50 mg/kg b.w.)	111.52±8.49	129.85±9.89[c]
5	Mangiferin-Alonem (50 mg/kg bw)	113.13±8.66	136.15±10.42[a]

Values are expressed as mean±SD for 6 hamsters in each group. Values not sharing a common superscript letter differ significantly at [(a-c)] $p < 0.05$.

Statistical analysis

The data were illustrated as a mean ± standard deviation. Biochemical parameters were executed with one-way ANOVA afterward Duncan's Multiple Range test (DMRT) for the comparison by statistically. The results were judged statistically significant if $P < 0.05$.

RESULTS

Effect of mangiferin on body weight of hamsters

The b.wt was precised as changes involving, starting, and finishing period of control and treated hamsters are depicted in Table 1. Significant ($P < 0.05$) depletion on b.wt was noted in DMBA-induced OSCC, while supplement with 50 mg/kg b.wt of mangiferin by orally in thrice times for each week for up to 14 weeks exhibited a significant ($P < 0.05$) improvement in b.wt gain of DMBA challenged hamsters. Orally, pre-administration of mangiferin alone hamsters revealed a similar b.wt when matched with the untreated normal group.

Incidence of oral tumor

Incidences, volume and TB of tumors of normal and studied hamsters were depicted on Table 2. In our work, 100% tumor development noted by way of mean tumor volume (189.37 mm^3) and TB (1704.33 mm^3) in DMBA induce HBPC. Mangiferin with DMBA treated hamsters significantly ($P < 0.05$) undeveloped the incidence, volume, and burden of tumors. Untreated normal hamsters possessed no tumor formation as well as mangiferin alone hamsters.

Histopathological evaluation oral mucosa tissue

The pathological changes observations of the oral mucosa of normal and studied hamsters are illustrated in Figure 2 and Table 3. In our work, 100% development of tumor and harsh hyperkeratosis, hyperplasia, dysplasia, and well-documented OSCC were observed in the cheek pouch epithelium of tumor growth HBP. While well-differentiated squamous cell carcinoma (SCC) was not observed on buccal pouch epithelium of DMBA treatment with mangiferin administered hamsters were moderate, mild keratosis and hyperplastic epithelium. Oral administration of mangiferin alone and untreated control exhibited clear epithelial deposits.

Status of plasma and oral mucosa lipid peroxidation

Figure 3 explains the level of LPO derivatives of TBARS, CD, and LOOH on plasma and cheek pouch of normal and treated hamsters.

Table 2. Tumor incidence, tumor number, tumor volume and tumor burden of control and experimental hamsters.

Groups/ Treatment	Control	DMBA	DMBA + Mangiferin	Mangiferin -Alone
Tumor incidence	-	100%	-	-
Total number of tumor/hamsters	-	9±0.69	-	-
Total volume (mm^3)/hamsters	-	189.37±14.42	-	-
Tumor burden (mm^3)/hamsters	-	1704.33±130.52	-	-

Values are expressed as mean±SD for six hamsters in each group. Values not sharing a common superscript letter differ significantly at [(a-c)] $p < 0.05$.

Table 3. Histopathological changes in the oral mucosa of control and experimental hamsters.

Groups/Treatment	Control	DMBA	DMBA + Mangiferin	Mangiferin -Alone
Keratosis	-	+++	++	-
Hyperplasia	-	+++	+	-
Dysplasia	-	+++	-	-
OSCC	-	+++	-	-

This work revealed the significant augmentation in LPO on plasma and suppressed in the buccal tissues of DMBA challenged hamsters when checked with normal animals. Supplementation of mangiferin by orally to DMBA-challenged hamsters exhibits near normal level of prominence LPO derivatives in plasma and cheek pouch when checked with the normal group, whereas control and mangiferin alone supplemented hamsters possessed no differences.

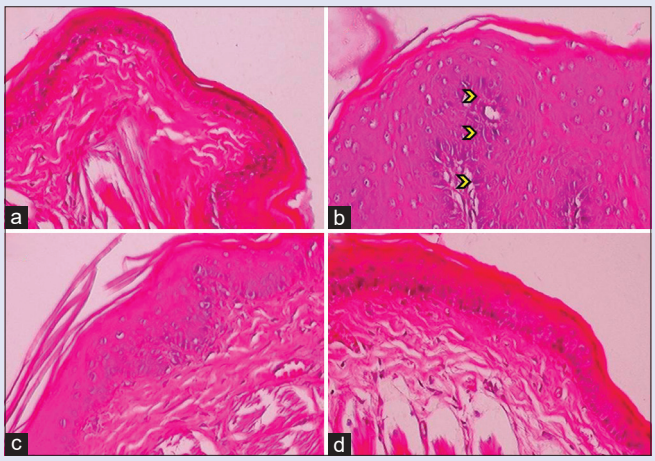

Figure 2: Histopathological changes in the oral mucosa of control and experimental hamsters. (b) Photomicrograph showing well differentiated oral squamous cell carcinoma exhibiting enlarged cells in Group 2 DMBA alone hamsters (arrow indicated). (c) Oral mucosa epithelium from Group 3 (DMBA + Mangiferin) hamsters showed normal cellular structural design by mild-to-moderated hyperkeratosis and hyperplasia. (d and a) Group 4 (Mangiferin – Alone) and Group 1 (control) hamsters showed normal squamous epithelium through no signs of cellular growth

Enzymatic antioxidant levels of plasma and oral mucosa

Figure 4 demonstrates the levels of enzymatic antioxidant on plasma and oral mucosal tissue of normal and studied hamsters. Enzymatic antioxidant status on plasma and cheek pouch was demonstrated the significant decreased, except for GPx (enlarged) in the oral pouch of hamsters OC. Oral administered with mangiferin to DMBA-challenged hamsters confirmed the significant ($P < 0.05$) brought back to the near normal level of antioxidant enzymes as compared to the untreated control; however, mangiferin supplemented hamsters demonstrate nil significant variation on the enzymatic antioxidant activity as compared with untreated normal group.

Levels of plasma and cheek pouch non-enzymatic antioxidant

Figure 5 illustrates the non-enzymatic antioxidants level of plasma and cheek pouch of untreated normal and treated hamsters. Considerable decrease of non-enzymatic antioxidants of plasma was noted, while they augmented in the cheek pouch of hamsters during OSCC. Pre-administered with mangiferin to DMBA-challenged hamsters revealed significant near normal levels of non-enzymatic antioxidants to both plasma and buccal mucosa when checked with the untreated normal group; additionally, hamsters treated with mangiferin alone and untreated control possessed none modifications in non-enzymatic antioxidants function.

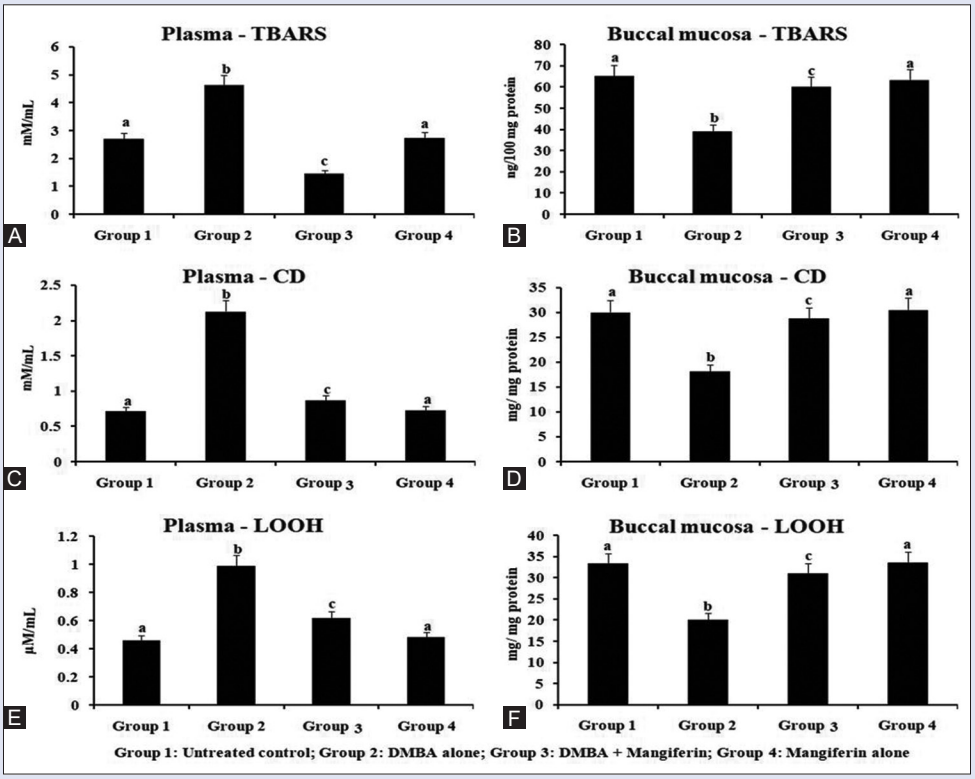

Figure 3: The status of lipid peroxidation by-products in the plasma and buccal mucosa of control and experimental hamsters. A,C,E) Plasma - TBARS, CD and LOOH, B,D,F) Buccal mucosa - TBARS, CD and LOOH levels in experimental hamsters. Results are expressed as mean ± standard deviation for six hamsters in each group. Data not sharing a common superscript letter (a-c) differ significantly at $P < 0.05$ (DMRT)

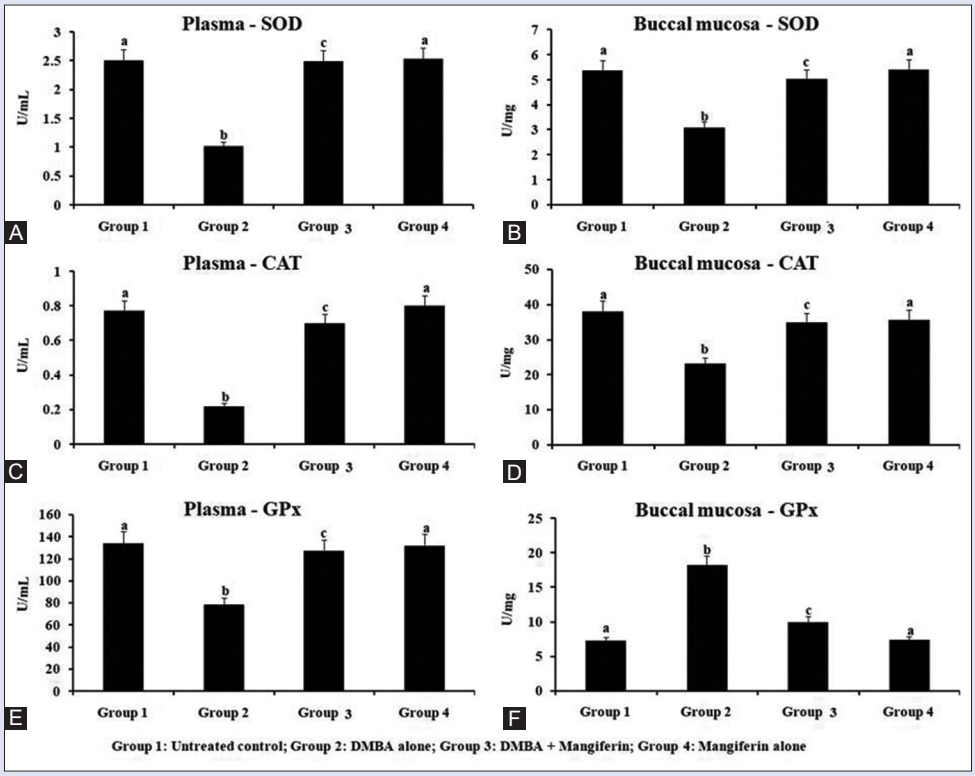

Figure 4: The levels of enzymatic antioxidant in the plasma and buccal mucosa of control and experimental hamsters. A,C,E) Plasma - SOD, CAT and GPX, B,D,F) Buccal mucosa - SOD, CAT and GPX levels in control and experimental hamsters. Results are expressed as mean ± standard deviation for six hamsters in each group. Data not sharing a common superscript (a-c) differ significantly at $P < 0.05$ (DMRT)

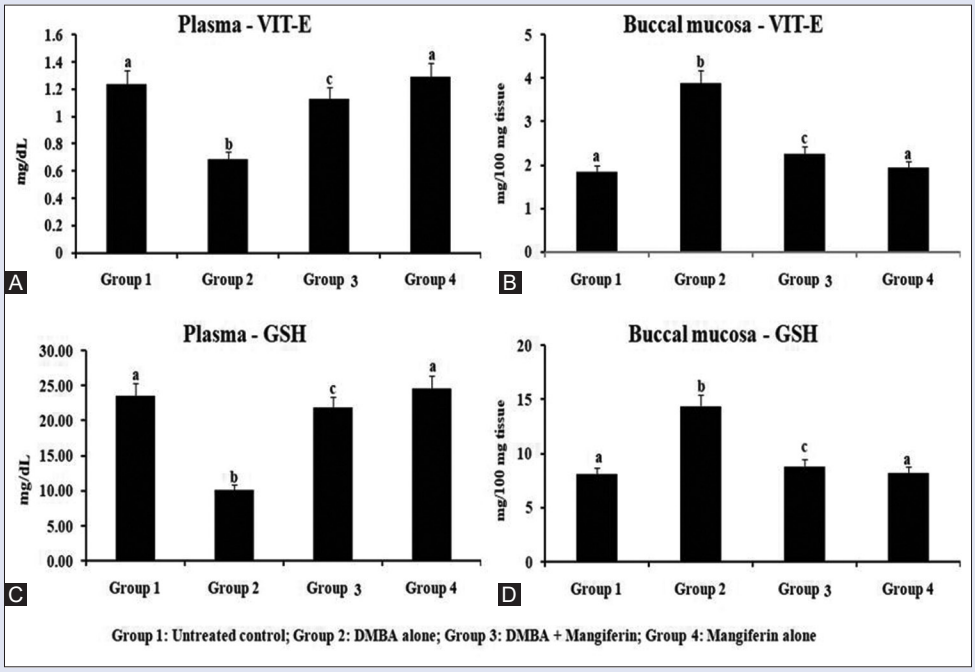

Figure 5: The status of non-enzymatic antioxidants in the plasma and buccal mucosa of control and experimental hamsters. A,C) Plasma – VIT-E and GSH, B,D) Buccal mucosa - VIT-E and GSH expression in experimental hamsters. Results are expressed as mean ± standard deviation for ten hamsters in each group. Data not sharing a common superscript (a-c) differ significantly at $P < 0.05$ (DMRT)

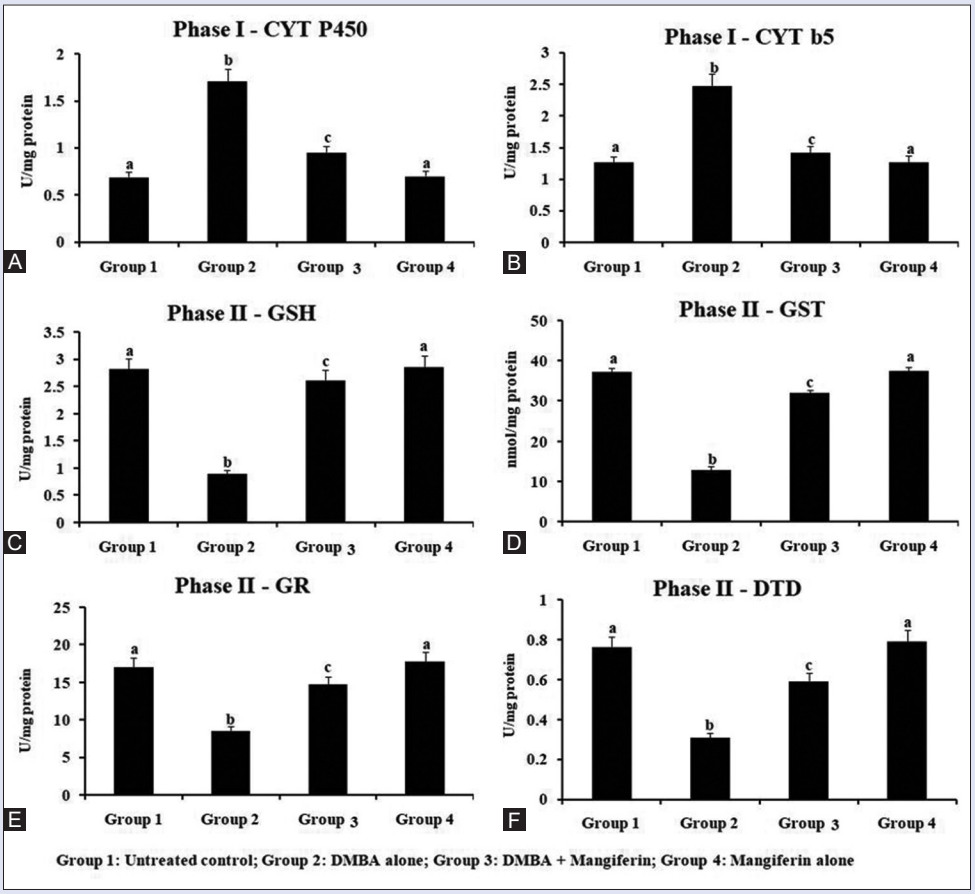

Figure 6: The status of Phase I and Phase II detoxification enzymes in the liver of experimental hamsters. A,B) Phase I – CYT p450 and CYT b5, C – F) Phase II – GSH, GST, GR and DTD levels in control and experimental hamsters. Results are expressed as mean ± standard deviation for six hamsters in each group. Data not sharing a common superscript (a-c) differ significantly at $P < 0.05$ (DMRT)

Detoxification marker enzymes level in hepatic tissue

Figure 6 revealed the Phase-I and Phase-II hepato-protective enzymes level in liver tissue of untreated control and treated hamsters. In this study, we detected that the Phase-I enzyme was enhanced, whereas Phase-II enzyme was considerably reduced in HBP tumor formation, but supplementation of mangiferin by orally to DMBA-challenged treated hamsters remarkably reinstated the Phase-I and II enzyme. Therefore, mangiferin supplemented hamsters revealed no notable alterations on Phase-I and II enzyme levels as contrast to untreated control.

Oral mucosal level of Phase I and II enzymes

Level of Phase-I and II detoxification enzymes of oral mucosa of untreated normal and treated animals is depicted in Figure 7. Functions of Phase-I detoxification mediators were remarkably enhanced, whereas Phase-II altered (GSH/GSSG proportion was elevated; GSSG was suppressed) on HCP carcinogenesis. Supplementation of mangiferin by oral route to DMBA-challenged hamsters notably regained the enzymatic functions of Phase-I and II detoxification regulators; though mangiferin in alone supplemented animals possessed nil variations on Phase-I and II enzymes level.

DISCUSSION

OC is a major health consequence among the Asian countries and regarded as the widespread neoplasm condition that possessing a huge deleterious effect on well-being with elevated morbidity and mortality rate.[34] Chemoprevention technique was emerged as a preventing and treating of malignancies with the aid of natural-based herbal constituents.[35] *In vivo* chemopreventive and anticancer activities on mangiferin on various cellular and experimental models, it can explore through the inhibition of tumor growth in different treated hamsters models of various cancers. Li *et al.*[36] confirmed that the anti-neoplastic activity inhibition of cell viability and diminishing metastatic stages. DMBA-induced HBPC was adapted to measuring the chemopreventive capabilities of naturally occurring constituents; because DMBA-stimulated mouth carcinoma was narrowly exhibiting the mouth carcinoma of humans by histopathologically and as well as morphologically. The current research, a typical administration of DMBA for 14 weeks regimen possessed a well-built SCC and linked with the elevated burden of tumor and also exhibited harsh hyperplasia, hyperkeratosis, and dysplasia. This study revealed the 100% formation of tumor on buccal pouches of DMBA challenged hamsters only, while tumors were histopathologically confirmed with slightly altered squamous cell carcinogenicity.

Tan *et al.* 2018[12] have been reported that mangiferin administrated through orally inhibited orthotopic hepatocellular carcinoma growth in implantation dose-dependently suppressed the free expansion in experimental and invasion *in vitro* model via β-catenin in Wnt pathway. In the present study, supplementation of 50 mg/kg b.wt of mangiferin by oral route to DMBA-challenged hamsters remarkably inhibited and suppressed tumor development. Consequently, our findings signify that mangiferin have significant chemopreventive efficiency while

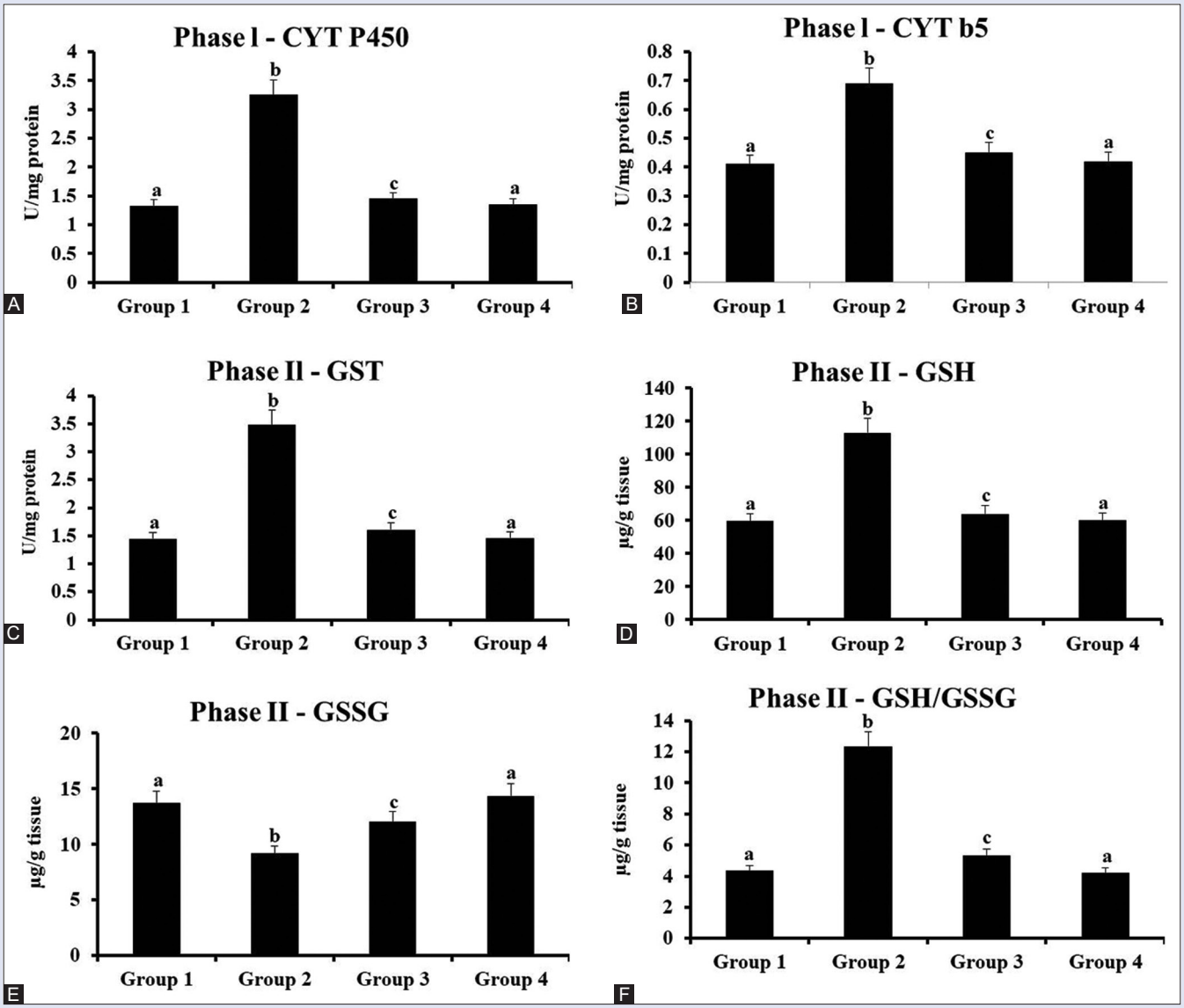

Figure 7: The status of Phase I and Phase II detoxification enzymes in the oral mucosa of experimental hamsters. A) CYT-p450, B) CYT-b5, C) GST, D) GSH, E) GSSG and F) GSH/GSSG expression in control and experimental hamsters. Results are expressed as mean ± standard deviation for six hamsters in each group. Data not sharing a common superscript (a-c) differ significantly at $P < 0.05$ (DMRT)

DMBA mediated mouth malignancy. Chemopreventive prospective of mangiferin may because of its destructive action against neoplasm condition through mouth malignancy.

A chemo-preventive regulator altered the DNA damaging units to the removable metabolites via excretion by means of instigation of detoxification mediators.[37] Rajendran *et al.* 2008,[38] A, and Rajendran *et al.* 2013,[39] B, have been postulated that mangiferin (50 mg/kg b.wt) delayed the tumor developments in mice with no notable alterations of the body mass. Rajendran *et al.*[40] (C) and Hu *et al.*,[41] have been found that pretreatment of mangiferin (50 mg/kg b.wt) for 5 weak in the Swiss albino mice model in this due explored the suppression tumor growth development. These studies were corroborating with our findings. Shi *et al.* 2016[42] have been revealed that mangiferin can kindle up G2/M phase cell cycle detain throughout downregulating Cdc2-cyclin B1 and induces apoptosis through suppressing human lung cancer cell lines. Further, in their studies, mangiferin exerts antineoplastic effects experimental animals, with more potent to drastically diminish the burden and volume of subcutaneously and enlarge A549 xenograft of mice span. Additional work on mangiferin revealed the scavenging efficiency of mangiferin which may benefit to guard the cells besides oxidative stress stimulated injury and mutagenesis. Schwartz and Shklar,[43] established that mediator which stimulate the enzymatic functions of GST possess remarkable chemo-preventive prospective while the formation of cancer. Diminished functions of Phase-II toxins excreting stimulator were accounted for numerous varieties of cancer development stages in order.[44] Hence, the present study confirmed the orally administered mangiferin with DMBA-treated hamsters come back the level of Phase-II toxins removing regulators up to near normal level. The current work disclosed that the mangiferin improved removal and eliminating processes of metabolites of malignant cells while DMBA-challenged HBPCs.

Generation of reactive oxygen species (ROS) and elevated oxidative stress takes a major role in the pathological process of different malignancies together with OC. Assessment of TBARS level in serum of plasma was an unfailing indicator to measure the injury level of

tissues under pathophysiological circumstances. Although GST and GR were the detoxify DMBA metabolites, further escaped diol epoxide derivatives able to attach with adenine deposits of DNA which causes mutation which endorses the endurance and progression of cells. Magniferin at dose 100 mg/kg b.wt, medicated mice (C57BL/6J) was confirmed the development of tumor than cisplatin, which was explored the mangiferin was good chemopreventive mediators.[45] This study was similar to our findings. TBARS of DMBA-challenged hamsters revealed the LPO depleting potential of mangiferin while mouth malignancy. Peng *et al*. 2004[46] reported the suppressive efficacy of mangiferin against blood cancer (k562) cell proliferation, also downregulated the nuclear factor kappa B (NF-kB) induces programmed cell death. Rajendran *et al.* 2014[40] suggested that mangiferin strong anticancer and potent to chemopreventive agents due to their between the suppression and additional accumulation of free radicals and depleted the incidence of cancer.

Improved LPO connected by the way of depleting the antioxidants on passage was a key verdict on the conversion of malignancy. ROS discharge extremely toxic oxygen molecules, navigate layers and stimulate negative property of this position apart from carcinogenesis.[47] The better LPO in the passage of hamsters challenged with DMBA mouth tumors imitates too much accumulation of free radicals aggravated by diminished competence of defense mechanisms of hosts. The elevation on LPO directly interlinked to the reduction of enzymatic antioxidants. The ascorbic acid (Vitamin-C) is a major and very important antioxidant that vanishes quicker than any other antioxidants at the time of exposure of plasma to elevated ROS.[48] Vitamin-E is an imperative antioxidant and also a lipid solubilizing agent occurs in the blood and mucosabuccal tissues.[49] GSH, an important *in vitro* reluctant, presents defence besides the free radicals, peroxides, and other toxic agents.[50] Insufficiency of Vit-C, Vit-E, and GSH in the blood of tumor having animals might be owing their improved consumption to forage of produces of LPO. The depleted enzymatic functions of GPx, SOD, and CAT which are essential toxins removing enzymes of cells was described in carcinogenicity.[51] These findings were narrowly supported the current work. Supplementation of mangiferin through oral route was regained the alterations which are mediated by DMBA and this finding highlighting the suggestions of chemopreventive efficiencies of plant-derived constituents.

Mangiferin was mentioned as alter the LPO level while generating the elevated antioxidants. It was already mentioned that the mangiferin can elevate the GSH, GPx, SOD, and CAT level.[52] The current work exhibited that mangiferin possessed the suppression of tumors through transforming the LPO and antioxidant levels on intent organs. Das and Roy[53] were studied that hepatoprotective effect, which is augmented LPO and lower the levels of cellular GSH in D-galactosamine (GAL)-stimulated hepatotoxicity rats model. Further, in his studies showed in the *in vitro* study liver cells exposed GAL5 mM were stimulated the apoptotic condition and cell death with elevated ROS and NO accumulation. Rajendran *et al*.[38] have been described that oral administrated mangiferin proved excess production of the detoxification regulators, such as GSHtransferase, quinine reductase and uridine 5'-diphosphate-glucoronosyl transferase, hold back the genotoxicity in lung-bearing rats.

The above described findings revealed that mangiferin able to be an effectual chemotherapy drug and by additional investigations on mangiferin can endorse the prevention and treatment of malignancies. ROS has to be removed quickly to evade the cell injuries and necrosis and carcinogenesis as well, this was mediated by the antioxidants via regulation of toxin removing pathway. In this study, mangiferin proved as a recognized antioxidant drug that can neutralize and remove the widespread ROS because of the influential expressions, functions as important toxins removing agents supplied to depletion in oxidative stress. It was previously mentioned that the mangiferin narrowly defends against elevated ROS levels mediated by widespread agents.[54] Alongside its free radical scavenging potential, mangiferin power to ROS production by traversing fenton-type responses.

Fenton-type responses normally engaged in the hydroxyl radical accumulation and thereby oxidation of Fe^{2+} to Fe^{3+}. With the mangiferin treatment, Fenton-type responses were refused via connecting the Fe^{2+} ions and by restrain the ROS accumulation.[55] Furthermore, Duang *et al*.[56] have done that mangiferin protects against LPO. This defense mechanism might within part accountable for depleted DNA adduct formation and attenuation of cytotoxic functions. Mutually cellular and experimental confirmations recommend the improved expressions of widespread toxins removing enzymes mediated by mangiferin lead to attenuation of ROS accumulation. In supplement to these findings,[9] mangiferin prejudiced CAT, SOD, and GPx in that order it halt the ROS centered apoptosis via depleting the intercellular accumulation of ROS. Banerjee *et al*.[57] revealed the relationship within the mangiferin and GSH through arrangement by mangiferin elevated excessive level of GSH in experimental supplementation with erstwhile antioxidant agents. Sarkar *et al*.[58] additionally recommends the efficiency mangiferin to vanish the elevated oxidative stress was may be connected with downregulation of NF-kB, which depletes the tumor necrosis factor (TNF)-mediated ROS accumulation. CAT is an essential toxin removing enzyme responsible for antioxidant ability in many organisms possessing oxygen which renovates H_2O_2 to H_2O and O_2. If H_2O_2 was not quickly removed or converted to tiny species, it could stimulate the oxidative injury. Mangiferin was directly escalating the CAT enzyme's efficacy via mutual functions with the enzyme, thereby depleting the oxidative injury that can able to complete in earlier to the removal of H_2O_2. The escalated function of CAT could vary downstream pathways which approval an atmosphere does not endorse carcinogenicity.[59]

Leiro *et al.* 2003[60] have demonstrated that mangiferin inhibits the inducible nitric oxide synthase and TNF-α gene expression which exhibits mangiferin have therapeutic of inflammatory and hemo-generative disorder. Yoshimi *et al.* 2001[61] showed 50 mg/kg b.wt., to have *in vivo* tumor growth suppressive action against AZO enzyme induced rats in colon cancer model due to their anti-cell proliferative activity this property were strongly recommends a mangiferin was potential naturally occurring chemopreventive drug. Cuccioloni *et al.* 2016,[62] are postulated that mangiferin has a therapeutic potential to selectivity block breast cancer cell multiplication through striking the multiplication of cells and stimulating apoptosis. These findings were more supported to our present finding suggested on mangiferin have anti-cancer, anti-neoplastic, and anti-metastatic properties.

CONCLUSION

Taken together, the present study thus concludes that protective effect of mangiferin on tumor cell proliferation in DMBA-induced HBPC. While the mechanism through which mangiferin exerts its prevented DMBA induced OC in the HCPvia its anti-cell proliferative anti LPO and antioxidant possible and also adjusting the prominence of Phase-I and Phase-II hepatoprotective mediators. This study was supportive in determining the dose employed against oral tumours in the development of a new and a potential anticancer drug. It may offer a drug to use in clinical phases needs more investigations on its the molecular means of functions and probable usefulness of mangiferin as an drug for chemotherapy.

Acknowledgements

The authors would like to thank Henan Provincial Science and Technology Department Project, 172102310613.

Financial support and sponsorship

Henan Provincial Science and Technology Department Project, 172102310613.

Conflicts of interest

There are no conflicts of interest.

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