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Research

Role Of Oldenlandia Umbellate On Membrane Bound Enzyme Activity In Den Induced HCC

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	Abstract
Published on: 06 May 2025	Hepatocellular carcinoma (HCC), one of the most prevalent and deadly liver cancers globally, is often triggered by various chemical carcinogens such as diethylnitrosamine (DEN). This study explores the chemopreventive role of <i>Oldenlandia umbellata</i> on membrane-bound enzyme activity in DEN-induced hepatocarcinoma in male Wistar rats. The research aimed to evaluate the efficacy of the methanolic extract of <i>Oldenlandia umbellata</i> in modulating liver function markers and the activities of vital ATPases Na^+/K^+, Ca^{2+}, and Mg^{2+} ATPases damaged by DEN exposure. The experimental setup included three groups: control, DEN-induced, and DEN + <i>O. umbellata</i>-treated rats. The treatment group received a dose of 250 mg/kg of <i>O. umbellata</i> extract for 45 days. Biochemical assays showed significant elevation in liver function markers and a reduction in ATPase activity in DEN-treated rats. However, treatment with <i>Oldenlandia umbellata</i> significantly reversed these alterations, restoring enzyme activity and normalizing serum markers. Histopathological observations supported the biochemical findings, showing improved liver architecture in treated groups. These results suggest that <i>Oldenlandia umbellata</i> possesses potent hepatoprotective and antioxidant properties, possibly due to its ability to stabilize cellular membranes and reduce oxidative stress. The study concludes that the plant extract holds promise as a natural therapeutic agent against liver carcinogenesis.
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Creative Commons Attribution 4.0 International License.	Keywords: <i>Oldenlandia umbellata</i>, Hepatocellular carcinoma (HCC), Diethylnitrosamine (DEN), ATPase enzyme activity, Liver function tests, Antioxidant and hepatoprotective effects.

INTRODUCTION

Tumours can be categorized clinically and morphologically into benign and malignant on the basis of certain characteristics as rate of growth, cancer phenotype and stem cells, clinical and gross features, microscopic features, local invasion and metastasis. It has become a central issue in medicine as it is the foremost cause of death in economically developed countries and the second leading cause of death in developing countries. All cancers are multi-factorial in origin; they include genetic, hormonal, metabolic, physical, chemical and environmental factors (Mohan, 2015;) With the advancements in modern surgical and non-surgical remedial modalities significant reduction in cancer mortality is observed, but continuing search for better control and protective methods are needed in order to reduce the cancer mortality and related side effects¹. Hence, there is a continuing need to search the naturally occurring compounds in modern and traditional system of medicine as a novel therapeutics, which either suppress or prevent the process of carcinogenesis (Thapliyal *et. al.*, 2022).

Hepatocellular carcinoma (HCC), also called malignant hepatoma is the most common primary liver cancer which accounts as the third leading cause of deaths worldwide, after lung and stomach cancer. It is responsible for a worldwide incidence of over one million cases annually. The HCC, a fatal malignancy extends in several regions of Africa and Asia (80%). The majority of HCC cases attributable to primary infections are induced by hepatitis B and C viruses, aflatoxin exposure, cirrhosis, environmental pollutants, obesity, iron overload, and nitrosamines.

The well-known hepatocarcinogen, diethylnitrosamine (DEN) a representative compound of the nitrosamine family has been suggested to cause the generation of reactive oxygen species (ROS), forming DNA carcinogen adducts in the liver and results in oxidative stress and cellular injury². It has been widely reported to be found in the environment, in tobacco smoke, varieties of foods as cheese, salted and dried fish, soyabean, cured meat, alcoholic beverages, and ground water having high level of nitrates making the human population vulnerable to its exposure, and is also synthesized endogenously. Hence, DEN has been used as an effective experimental model in the field of carcinogenesis and chemoprevention.

ATPases, the membrane-bound enzymes are very sensitive to peroxidation reactions and are closely associated with the plasma membrane which participates in the energy requiring translocation of sodium, potassium, calcium and magnesium ions. These enzymes regulate ion transport across cellular membrane, cellular volume, osmotic pressure and membrane permeability. Na^+/K^+ ATPase (also known as the Na^+/K^+ pump) is an enzyme located in the plasma membrane that pumps three sodium ions out of the cell for every two potassium ions pumped³. The Ca^{++} ATPase pump in plasma membrane because of its high affinity has been proposed as the structure responsible for the maintenance of calcium concentration at the submicromolar level. Mg^{++} is required for maintaining the cell cycle cytoskeleton and mitochondrial integrity, protein and nucleic acid synthesis, and for the binding of substrates to the plasma membrane. It frequently modulates ion transport by pumps, carriers and channels, and thereby may modulate signal transduction. Activities of these membrane-bound enzymes are affected with hepatic injury resulting from peroxidation of membrane lipids (by alteration of structural and functional characteristics of the membrane) (Senthilnathan *et. al.*, 1987; Kannampalli *et. al.*, 2007;).

In recent years, a large number of natural compounds have been identified and shown to have potential cancer chemo preventive significance. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various ailments including cancer (Balachandran and Govindarajan, 2015).

The liver is the largest organ of the human body. It is dark reddish-brown, shape like a triangle weighing close to three pounds (about 1.4 kg). It is located behind the ribcage on the upper right quadrant of the abdomen^{4,5}.

MATERIALS & METHODS

Chemicals

- ❖ Diethylnitrosamine (DEN), Cellulase and Pectinase of *Aspergillus niger* were purchased from Sigma Aldrich Chemical Company (Bangalore).
- ❖ ALP, AST, ALT, and Bilirubin (Total and Direct) kits were procured from Erba Diagnostics.
- ❖ SP twin electrolyte, Calcium, and Magnesium were purchased from Span Diagnostic Ltd.
- ❖ Adenosine triphosphate (ATP) was procured from Himedia. Pvt Ltd Mumbai.

All other chemicals (solvents and reagents) used in this study were of analytical grade of S.D. Fine Chemicals.

Extract Preparation

One kg of whole plant of *Oldenlandia umbellata* was shade dried coarsely powdered and soaked in methanol in aspirator bottles and exhaustively extracted at room temperature for 72 hrs. The solvent was decanted, filtered and distilled off in Rotovac apparatus. The methanol extract was completely dried from solvent under reduced pressure using high vacuum conditions. The collected extracts were then taken up for further investigations⁵².

Methods

Animal Selection and Housing

Adult male albino rats (wistar) weighing 120-200 g were selected for chemoprevention of hepatocellular carcinoma activity. They were procured from Venkateshwara Enterprises, Bangalore. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at $(27 \pm 2)^\circ\text{C}$ under 12 h dark / light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water *ad libitum* was provided. Ethical clearance for use of the animals was obtained from the institutional animal's ethical committee Ratnam institute of pharmacy prior to the beginning of the project work^{53,54}.

Handling of carcinogen

The carcinogenic material diethylnitrosamine (DEN) procured from Sigma-Aldrich chemicals was handled carefully as suggested by the supplier and according to the Standard operating procedure (SOP)^{55,56}. The DEN containing vial was placed in a plastic freezer bag and stored in the freezer at -20°C . While using, the vial was taken to the fume hood and a diaper was placed over the work area. Gloves were worn and required amount of DEN was collected directly through syringe. The vial with the chemical was weighed and the quantity of chemical determined by subtraction. Contact materials were disposed in the infectious/biohazard waste⁵⁷. Unused solutions were arranged for proper disposal and incineration.

Qualitative chemical analysis (Kokate *et. al.*, 2009; Khandelwal, 2008): The enzymatic extract of *Oldenlandia umbellata* was subjected to qualitative chemical analysis to detect the presence of various phytoconstituents.

Treatment Protocol

Table 1: Treatment protocol

Group	No. of animals	Status	Treatment
I	6	Vehicle control	Receives 0.9% w/v normal saline daily for 45 days (Pradeep <i>et. al.</i> , 2010).
II	6	Tumour control DEN	Received DEN at a single dose of 200 mg/kg (body weight) intraperitoneally on 1 st week followed by administration drinking water up to 45 days (Pradeep <i>et. al.</i> , 2010).
III	6	DEN+ <i>O.U</i> (250)	Received DEN at a single dose of 200 mg/kg (body weight) intraperitoneally on 1 st week followed by administration of drinking water up to 45 days. <i>Oldenlandia umbellata</i> was given orally after 2 nd week (5 day/week) at a dose of 250 mg/kg (body weight) up to 45 successive days (Wang <i>et. al.</i> , 2010; Pradeep <i>et. al.</i> , 2010).

Preparation of DEN

DEN (single dose at 200 mg/kg) was emulsified in 0.9% NaCl for the experiment (Sivalokanathan *et. al.*, 2006).

Dose preparation

For the experiment, enzymatic extract of *oldenlandia umbellata* (250 mg/kg) was dissolved in 0.9 % NaCl and given orally.

Collection of blood and organs

The animals were anaesthetized under mild anaesthesia at the end of the study (after 16 weeks for tumour promotion and 45 days for tumour initiation model). Blood samples were collected by retro-orbital puncture for serum biochemical parameters and were centrifuged to get clear serum. The animals were sacrificed by cervical dislocation and livers were dissected out and 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer (pH 7.4) and was used for the assay of various activities as membrane bound enzyme and DNA and RNA estimation by standard methods. Further the livers dissected out was blotted off from blood, washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group respectively^{67,68}.

Standard curve for estimation of membrane bound ATPase

Table 2: Absorbance of different concentration of standard phosphorus

SL. No.	Concentration of Phosphorus (µg/ml)	Absorbance (at 620 nm)
1	0	0.003
2	4	0.08
3	8	0.165
4	12	0.259
5	16	0.339
6	20	0.414
7	24	0.484
8	28	0.566
9	32	0.638
10	36	0.708
11	40	0.789
12	44	0.864
13	48	0.938
14	52	1.021
15	56	1.12
16	60	1.226
17	64	1.296
18	68	1.386
19	72	1.474
20	76	1.586
21	80	1.676

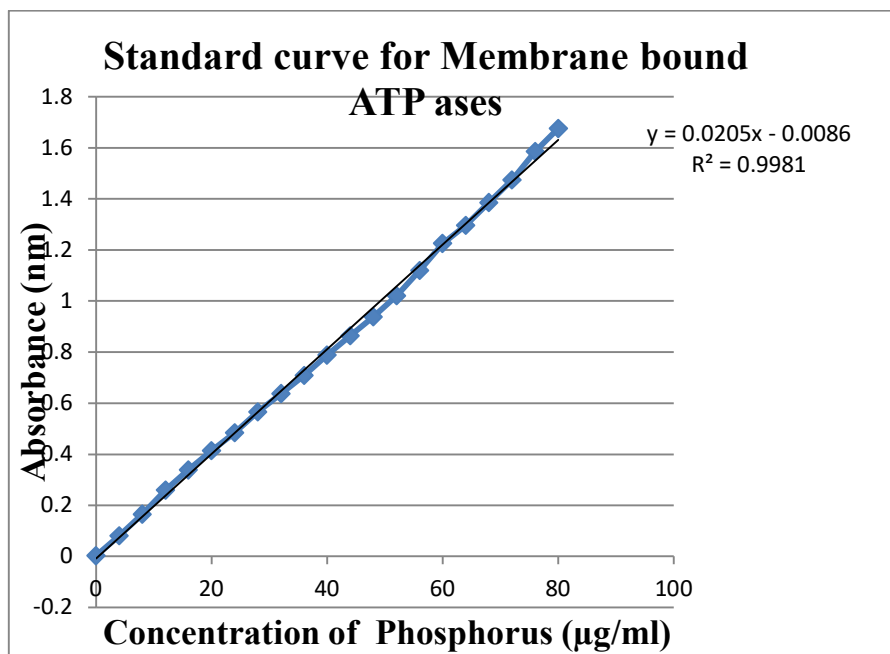


Fig 1: Standard curve for Membrane bound ATPase

Statistical analysis

All data are expressed as Mean $\pm$ S.E.M. The statistical significance was analyzed by using one-way ANOVA followed by Bonferroni's multiple comparison test^{81,82}. For statistical analysis, data was computed by using Graph Pad Prism Software version 5.0.

RESULTS

Liver weight

Animals administered with DEN showed significant increase in liver weight ($9.98 \pm 0.30, p < 0.01$) when compared with normal group (8.52 ± 0.20). The treatment group (*O.umbellatae*) significantly decreased the level of liver weight to ($9.02 \pm 0.22, p < 0.05$), when compared with DEN group.

Table 3: Liver weight (g) in various groups

SL. No.	NORMAL	DEN	DEN + O.U-250
1	9.23	9.98	8.68
2	9.01	10.67	8.90
3	8.15	10.98	9.06
4	8.35	9.06	8.96
5	7.98	9.85	10.06
6	8.45	9.35	8.46
Mean $\pm$ SEM	8.52 $\pm$ 0.20	9.98 $\pm$ 0.30**	9.02 $\pm$ 0.22 [#]

Values are expressed as Mean $\pm$ SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where ** $p < 0.01$, compared with normal group, and [#] $p < 0.05$, compared with DEN group.

Relative liver weight

Animals administered with DEN showed significant increase in relative liver weight ($4.74 \pm 0.18, p < 0.05$) when compared with normal group (3.65 ± 0.24). The treatment group (*O.umbellatae*) significantly decreased the relative liver weight to ($3.82 \pm 0.24, p < 0.05$), when compared with DEN group.

Table 4: Relative liver weight (g %) in various groups

SL. No.	NORMAL	DEN	DEN + O.u-250
1	4.52	4.79	3.54
2	3.76	5.15	3.56
3	4.03	5.35	4.31
4	3.47	4.25	3.64
5	2.82	4.60	4.79
6	3.30	4.30	3.13
Mean $\pm$ SEM	3.65 $\pm$ 0.24	4.74 $\pm$ 0.18*	3.82 $\pm$ 0.24 [#]

Values are expressed as Mean $\pm$ SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where * $p < 0.05$, compared with normal group, and [#] $p < 0.05$, compared with DEN group.

Estimation of liver function test

DEN alone treated group showed a significant increase in ALT (IU/L), AST (IU/L), ALP (IU/L), total bilirubin (BIT) levels (mg/dL), direct bilirubin (BID) levels (mg/dL) when compared to normal group. *O.umbellatae* treated animals overcame the deleterious effects of DEN.

ALT levels

The ALT levels were found to be significantly increased ($158.5 \pm 9.40, p < 0.001$) in DEN treated group, when compared with normal group (46.47 ± 4.43). The treatment group (*O.umbellatae*) significantly decreased the serum ALT levels to ($69.63 \pm 9.42, p < 0.001$) when compared with DEN group.

Table 5: Serum ALT levels (IU/L) in various groups

SL. No.	NORMAL	DEN	DEN + O.U-250
1	51.80	120.85	90.00
2	62.41	182.50	97.00
3	48.09	163.51	51.27
4	48.09	145.56	47.74
5	34.00	158.50	84.00
6	34.40	180.16	47.74
Mean ± SEM	46.47 ± 4.43	158.5 ± 9.40***	69.63 ± 9.42###

Values are expressed as Mean ± SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where *** $p < 0.001$, compared with normal group, and ### $p < 0.001$, compared with DEN group.

AST levels

The AST levels were found to be significantly increased (359.6 ± 14.93 , $p < 0.001$) in DEN treated group, when compared with normal group (57.22 ± 4.76). The treatment group (*O.umbellatae*) significantly decreased the serum AST levels to (263.9 ± 20.03 , $p < 0.001$) when compared with DEN group.

Table 6: Serum AST levels (IU/L) in various groups

SL. No.	NORMAL	DEN	DEN + L.E-250
1	56.00	386.48	223.00
2	78.35	445.80	313.00
3	59.31	375.16	296.12
4	56.45	412.36	201.00
5	45.30	340.30	312.00
6	47.93	413.76	238.56
Mean ± SEM	57.22 ± 4.76	359.6 ± 14.93***	263.9 ± 20.03###

Values are expressed as Mean ± SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where *** $p < 0.001$, compared with normal group, and ### $p < 0.001$, compared with DEN group.

ALP levels

The ALP levels were found to be significantly increased (217.1 ± 6.45 , $p < 0.001$) in DEN treated group, when compared with normal group (116.6 ± 5.39). The treatment group (*O.umbellatae*) significantly decreased the serum ALP levels to (166.4 ± 13.46 , $p < 0.01$) when compared with DEN group.

Table 7: Serum ALP levels (IU/L) in various groups

SL. No.	NORMAL	DEN	DEN + L.E-250
1	120.00	212.45	158.00
2	129.00	225.12	216.00
3	118.38	196.15	145.13
4	105.22	215.15	142.01
5	96.80	210.50	199.00
6	130.12	243.16	138.19
Mean ± SEM	116.6 ± 5.39	217.1 ± 6.45***	166.4 ± 13.46##

Values are expressed as Mean ± SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where *** $p < 0.001$, compared with normal group, and ## $p < 0.01$, compared with DEN group.

BIT levels

The BIT levels were found to be significantly increased (1.39 ± 0.06 , $p < 0.001$) in DEN treated group, when compared with normal group (0.35 ± 0.06). The treatment group (*O.umbellatae*) significantly decreased the serum BIT levels to (0.64 ± 0.05 , $p < 0.001$) when compared with DEN group.

Table 8: Total bilirubin levels (mg/dL) in various groups

SL. No.	NORMAL	DEN	DEN + L.E-250
1	0.50	1.20	0.50
2	0.60	1.45	0.60
3	0.25	1.32	0.50
4	0.26	1.28	0.69
5	0.30	1.45	0.72
6	0.20	1.67	0.84
Mean ± SEM	0.35 ± 0.06	1.39 ± 0.06***	0.64 ± 0.05###

Values are expressed as Mean ± SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where *** $p < 0.001$, compared with normal group, and ### $p < 0.001$, compared with DEN group

BID levels

The BID levels were found to be significantly increased (0.64 ± 0.02 , $p < 0.001$) in DEN treated group, when compared with normal group (0.07 ± 0.008). The treatment group (*O.umbellatae*) significantly decreased the serum BID levels to (0.11 ± 0.008 , $p < 0.001$) when compared with DEN group.

Table 9: Direct bilirubin levels (mg/dL) in various groups

SL. No.	NORMAL	DEN	DEN + L.E-250
1	0.10	0.65	0.10
2	0.07	0.70	0.12
3	0.10	0.58	0.14
4	0.06	0.69	0.10
5	0.05	0.55	0.10
6	0.08	0.71	0.14
Mean ± SEM	0.07 ± 0.008	0.64 ± 0.02***	0.11 ± 0.008###

Values are expressed as Mean ± SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where *** $p < 0.001$, compared with normal group, and ### $p < 0.001$, compared with DEN group.

Estimation of membrane bound enzymes

Estimation of Na⁺/K⁺ ATPase

Activity of Na⁺/K⁺ ATPase was influenced by DEN, there was a significant decrease (0.76 ± 0.02 , $p < 0.001$) in its activity in DEN treated group when compared with normal group (2.06 ± 0.14). Significant elevation in the levels of Na⁺/K⁺ ATPase was observed in the treatment group (*O.umbellatae*) to (1.27 ± 0.02 , $p < 0.01$), when compared with DEN group.

Table 10: Na⁺/K⁺ ATPase (μmoles/min/mg protein) in various groups

SL. No.	NORMAL	DEN	DEN + L.E-250
1	2.39	0.80	1.29
2	1.51	0.75	1.26
3	2.31	0.74	1.39
4	2.38	0.69	1.29
5	2.05	0.81	1.22
6	1.76	0.82	1.18
Mean ± SEM	2.06 ± 0.14	0.76 ± 0.02***	1.27 ± 0.02##

Values are expressed as Mean ± SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where *** $p < 0.001$, compared with normal group, and ## $p < 0.01$, compared with DEN group.

Estimation of Ca⁺⁺ ATPase

Activity of Ca⁺⁺ ATPase was influenced by DEN which significantly decreased (0.68 ± 0.03 , $p < 0.001$) its activity compared with normal group (1.38 ± 0.04). Significant elevation in the levels of Ca⁺⁺ ATPase was observed in the treatment group (*O.umbellatae*) to (1.22 ± 0.08 , $p < 0.001$), when compared with DEN group.

Table 11: Ca⁺⁺ ATPase (μmoles/min/mg protein) in various groups

SL. No.	NORMAL	DEN	DEN + L.E-250
1	1.41	0.85	1.05
2	1.37	0.62	0.93
3	1.17	0.59	1.23
4	1.38	0.63	1.39
5	1.51	0.71	1.49
6	1.45	0.69	1.26
Mean ± SEM	1.38 ± 0.04	0.68 ± 0.03***	1.22 ± 0.08^{###}

Values are expressed as Mean ± SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where *** $p < 0.001$, compared with normal group, and ^{###} $p < 0.001$, compared with DEN group.

Estimation of Mg⁺⁺ ATPase

There was significant decrease (0.71 ± 0.02 , $p < 0.001$) in Mg⁺⁺ ATPase activity in DEN treated animal group when compared with normal group (1.63 ± 0.06). Significant elevation in the levels of Mg⁺⁺ ATPase was observed in the treatment group (*O.umbellata*) to (1.08 ± 0.07 , $p < 0.01$), when compared with DEN.

Table 12: Mg⁺⁺ ATPase (μmoles/min/mg protein) in various groups

SL. No.	NORMAL	DEN	DEN + L.E-250
1	1.64	0.75	0.94
2	1.53	0.69	1.25
3	1.55	0.70	0.84
4	1.89	0.63	1.28
5	1.45	0.73	1.03
6	1.74	0.80	1.15
Mean ± SEM	1.63 ± 0.06	0.71 ± 0.02***	1.08 ± 0.07^{##}

Values are expressed as Mean ± SEM (n=6), by one-way ANOVA followed by Bonferroni test. Where *** $p < 0.001$, compared with normal group, and ^{##} $p < 0.01$, compared with DEN group.

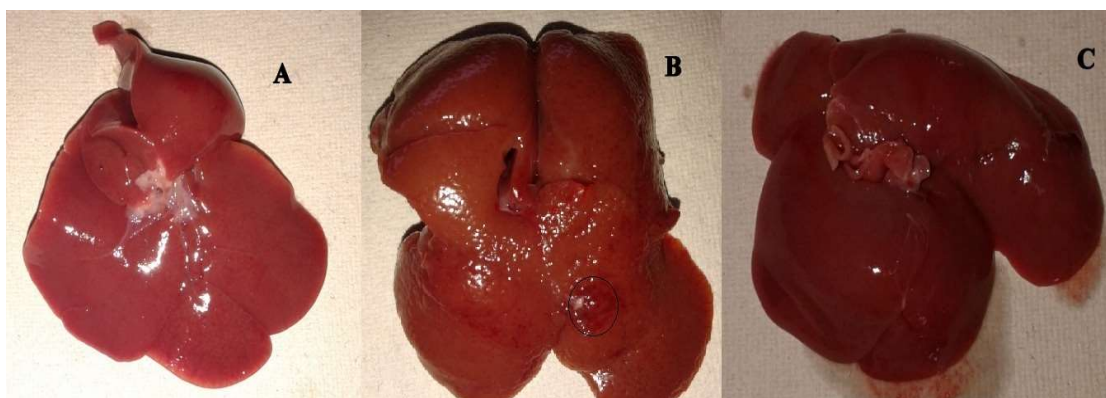
**Fig 2: Nodule formation**

Fig A: Normal liver showing no nodulogenesis.

Fig B: DEN treated liver showing nodulogenesis (circle).

Fig C: Treatment group (*Oldenlandia umbellata*) showing significant decrease in nodulogenesis when compared to DEN treated liver.

DISCUSSIONS

The aim of the present study was to investigate the role of *Oldenlandia umbellata* in diethyl nitrosamine (DEN) induced hepato carcinogenesis (HCC) in male albino rats. Diethyl nitrosamine (DEN), the most potent and

widely found chemical carcinogen, is used in animal models, which initiates and promotes liver cancer. DEN, a representative compound of the nitrosamine family has been known to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress and cellular injury^{83,84}(Bartsch and Montesano). The production of reactive oxygen species (ROS) in liver (main site of DEN metabolism) may be responsible for its carcinogenic effects which are shown to be metabolized to its active ethyl radical metabolite ($\text{CH}_3\text{CH}_2^\bullet$) and further interacts with DNA via different signal transduction pathways thereby producing mutation and oncogenesis (Bansal *et. al.*, 2015). This study was designed to study the chemopreventive effectiveness of *Oldenlandia umbellata* by its capacity to regulate membrane bound enzymes, tumour initiation parameters and liver function tests in DEN induced hepatocarcinoma.

General Observations

Previous reports suggest that DEN induced HCC decreases the body weight and increase the liver weight . Contrary to these reports, the DEN group showed significant increase in body weight when compared to control group. *Oldenlandia umbellata* treated group also showed significant increase in body weight when compared to DEN group. The DEN group showed significant increase in food consumption when compared to control group. On the other hand *Oldenlandia umbellata* treated group showed significant decrease in food consumption when compared to DEN group. As the data between the food consumption and body weight gain do not correlate with each other, the weight gain observed in treatment group could be independent of food consumption. This needs to be explored more to understand the reasons behind this phenomenon. Liver weight in DEN treated group was significantly increased which may be due to formation of tumour associated proteins which lead to the development of nodules and inflammation⁸⁵ (Sivaramakrishnan *et. al.*, 2018). *Oldenlandia umbellata* treated group significantly reduced the liver weight compared to DEN group. Relative liver weight was significantly increased in DEN group. This may be due to the increase in liver weight of cancer bearing animals. Significant improvement in relative liver weight was observed in *Oldenlandia umbellata* treated group. This may be due to the increase in the body weight although liver weights are normal.

Liver function tests

In the current study, administration of DEN significantly increased the serum levels of Alanine transaminase (ALT), Aspartate transaminase/aminotransferase (AST), Alkaline phosphatase (ALP) and total and direct bilirubin (BIT and BID). Previous studies have claimed similar rise in the levels of serum liver markers upon DEN administration. These markers are mainly engaged in the diagnosis of hepatic injury with HCC lesions and parenchymal necrosis where these marker enzymes are released from the damaged hepatocytes into the blood stream since these are primarily localized in liver . Alanine transaminase catalyses the transfer of amino group from alanine to α -ketoglutarate (α -KG) with the formation of glutamate and pyruvate. Serum Aspartate aminotransferase (AST) is a tissue enzyme that catalyses the exchange of amino and keto groups between alpha amino acids and alpha keto acids. The significant elevation in ALP level may be due to alteration in the membrane permeability leading to disarrangement in the transport of metabolites as this is membrane bound enzyme. Further, significant elevation of bilirubin level may be due to leakage of bilirubin into the circulatory system resulting from hepatocellular damage. This leads to permeability of liver membrane altering the buildup of unconjugated bilirubin in the blood. Treatment with *Oldenlandia umbellata* significantly reduced the levels of the serum markers compared to DEN group. This indicates the membrane stabilizing activity or the ability to repair the liver injury by maintaining the integrity of the plasma membrane, hence suppressing the leakage of enzymes through membranes, providing hepatoprotective action and inhibition of carcinogenesis.

Membrane-bound enzymes

The ATPases are membrane-bound enzymes which are vital in maintaining the homeostasis (ionic gradients between aqueous intra and extracellular phases) of metabolic needs of the body . The protection of the membrane-bound enzymes is indispensable in the treatment of diseases. The membrane-bound enzymes *i.e.*, Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase are responsible for the transport of sodium/potassium, magnesium and calcium ions across the cell membranes at the expense of ATP by hydrolysis. Previous reports of studies have shown that hepatic injury resulting from peroxidation of membrane lipids results in alteration of structural and functional characteristics of the membrane, thereby affecting the activities of the membrane-bound enzymes. These enzymes are susceptible to hydroperoxides and superoxide radicals, which might be the reason for their decreased activities during diethylnitrosamine administration. In the present investigation, it was observed that the decrease in the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase in liver tissues of DEN induced HCC animals may be due to increased production of free radicals leading to cell injury. Free radicals are clearly reported to mediate inhibition of membrane-bound ATPases by oxidizing the sulphhydryl groups in them . Damage of plasma membrane occurs directly through interaction with the membrane components such as the ion-dependent ATPases and ion channels and indirectly as a consequence of cytosolic damage. The inhibitory function of ion-dependent ATPases leads to disturbances in ion homeostasis resulting in impaired signal transduction and altered cellular

metabolism which further may cause alteration in cell membrane permeability, integrity and disturbances of vital function respectively. However, *Oldenlandia umbellata* treated rats significantly restored the tissue ATPase activities to near normal values. The results of this study reflects that *Oldenlandia umbellata* protects ion pump ATPases, apparently by limiting the degree of oxidation and levels of oxidation by products due to an increase in the reduced glutathione (GSH) content and increase in lipid peroxidation of the liver further demonstrating that *Oldenlandia umbellata* can modulate ATPase and reduce free radical formation in DEN-induced liver cancer in albino rats. Hence, the contents of *Oldenlandia umbellata* have the probability to minimize the deleterious effects of free radicals and can protect the structural integrity intimating efficacy of this agent against membrane damage tumorigenesis respectively.

CONCLUSION

Oldenlandia umbellata was studied for the hepatoprotective property which showed significant reduction in elevated serum markers (ALT, AST, ALP, total and direct bilirubin) compared to DEN group representing that it has the capability to repair the liver injury by preserving the integrity of the plasma membrane, thus suppressing the leakage of enzymes through membranes disclosing hepatoprotective action. The *Oldenlandia umbellata* protects ion ATPases, apparently by limiting the degree of oxidation and levels of oxidation by products thereby having the probability to minimize the deleterious effects of free radicals and can protect the structural integrity intimating efficacy of *Oldenlandia umbellata* against membrane damage tumorigenesis respectively and also improved levels of serum electrolytes. Further histopathological studies revealed that *Oldenlandia umbellata* treated liver tissues showed near normal architecture when compared with DEN group respectively. It can be concluded that *Oldenlandia umbellata* is having potential chemopreventive property as a food additive in hepatocellular carcinoma (HCC).

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