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# IN VIVO ANTICANCER SCREENING OF 'PORTULACA QUADRIFIDA LINN'ON COLON CANCER

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## ABSTRACT

Among all the carcinogenic diseases colon cancer is most commonly occurring cancer in developing countries. Many researchers had concluded that herbal source can be useful for the successful management of colon cancer. In this study we recruited a therapy with less side affects when compare to synthetic drugs using aqueous extract of *Portulaca quadrifida* a vegetative plant found in tropical parts of India by *In vivo* anticancer screening engrafted in male Sprague Dawley rats. In the view of characteristics of our work; For *In vivo* study, colon cancer was induced with the chemical carcinogen (DMH) and after treatment with the aqueous extract of *Portulaca quadrifida* for 16 weeks, the various *In vivo* parameters were evaluated. The *In vivo* antioxidant levels, carbohydrate metabolizing enzymes were increased in compound (200 and 400 mg/kg, p.o.) treated groups compared to control animals in a dose dependent manner. *In vivo* data thus obtained suggested that aqueous extract of *Portulaca quadrifida* is having good activity against the various biochemical parameters as compared with negative control group. The activity may be due to saponins content. Histopathalogical studies with aqueous extract of *Portulaca quadrifida* at 400mg/kg body weight showed no obvious abnormality in structure of colonic mucosa, which was very well comparable with biochemical, hematological, antioxidants and tumor markers estimation. With the above said findings we concluded that the plant *Portulaca quadrifida* posse's anticolorectal cancer activity, before its clinical usage, through toxicological profile has to be determined to confirm the safety of the drug.

**KEYWORDS:** *Portulaca quadrifida*, Colon cancer, *In vivo*.

## INTRODUCTION

The current position of low income countries shows direction towards the rise of chronic non communicable diseases such as cardiovascular disease, cancer and diabetes because of unhygienic lifestyle and diet.

Colon cancer is an important health concern worldwide and requires considerable attention in terms of disease management. Serious Chemotherapy and non steroidal Anti-inflammatory drugs (NSAIDs), improves the disease but they possesses many serious adverse effect so natural herbs are gaining importance. In this search directed to identify a plant material which will be useful and posses less side effect which will be beneficial in the treatment of colon cancer as we found a plant which is extensively grown in tropical parts of India as we have described in our previous research with *In vitro* method. [1] *Portulaca quadrifida Linn*. belongs to the family *Portulacaceae*. It is a small diffused, succulent, annual herb. It is used as a vegetable and also used for various curative purposes.[2]

## MATERIALS AND METHODS

Collection of Plant Material**:** Fresh whole plant material of *Portulaca quadrifida Linn* was collected from the local fields of Hyderabad. The plant specimen was identified and authenticated by Prof. Dr. (Mrs) Pratibha Devi, Department of Botany, Osmania University, Hyderabad. A voucher specimen is preserved in the herbarium of Department of Botany (Voucher No.024), Osmania University Hyderabad.

## EXTRACTION PROCEDURE

### PREPARATION OF AQUEOUS EXTRACT[3]

1. About 2000 gms of dried marc was taken in a 5000 ml of beaker and macerated with 3000ml of distilled water to which 100ml of chloroform was added as a preservative and kept it for seven days with occasional shaking daily in a closed vessel.

2. The supernatant was decanted and the marc was pressed then the pooled extract was concentrated on water bath at 50º c to get a dry solid mass. The percentage yield was calculated Results were tabulated in Table 1.

***In-vivo* anti-cancer screening by using rat model**

**1. Animal Study**

Male Sprague-dawley rats were purchased from The National Institute of Nutrition, Hyderabad. 300-400 gm body weight were used. All rats were kept at room temperature of 22+°C under 12 hr light/12 hr dark cycle in the animal house. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study. All animal procedures were performed in accordance with the recommendation of CPCSEA the proper care and use of laboratory animals the proposal of the present study was approved by IAEC of RVS College of Pharmaceutical Sciences, Coimbatore.

**Chemicals:** The 1,2-dimethyl hydrazine (DMH) was obtained from Sigma Chemical Company, Mumbai, India and all other chemicals and reagents used were of analytical grade.

**2. Preparation of DMH solution**

DMH was dissolved in 1 mm EDTA just prior to use and the pH was adjusted to 6.5 with 1 mm sodium bicarbonate to ensure the stability of the chemical.

**3. Induction of colon cancer:** Animals were given a weekly subcutaneous (s.c.) injection of DMH in the groin at a dose of 20 mg/kg body weight for 15 weeks[4]

**4. Preparation of 5 Fluorouracil solution (standard drug)**

The 5 Fluorouracil was dissolved in Normal saline.

**5. Preparation of drug sample**

The aqueous extract was weighed at the dose of 200mg/kg and 400mg/kg was dissolved in distilled water to provide a clear solution, which was administered to the animals through oral route.

**6. Treatment schedule**

After the administration of DMH, the animals were grouped into four groups of six animals in each. One group of animals was treated as control received normal saline only, out of three, one group receives standard drug and remaining two groups received 200mg/kg and 400mg/kg extract dose for 15 weeks.[5]

During the course of study individual animal body weight were recorded, alternate week till the end of the treatment.

**Results were tabulated in table 2.**

| Group | Treatment |
|---|---|
| Group I | Control +1 ml of Normal saline. p.o everyday for the entire period of the study. (Figure 1) |
| Group II | DMH (20mg/kg body weight once in a week for 15 weeks, s.c) (Figure 2) |
| Group III | DMH (20mg/kg body weight once in a week for 15 weeks, s.c) + 5 fluorouracil (20mg/kg) i.p (Figure 3) |
| Group IV | DMH (20mg/kg body weight once in a week for 15 weeks, s.c) + plant extract ( 200mg/kg), p.o daily (Figure 4) |
| Group V | DMH (20mg/kg body weight once in a week for 15 weeks, s.c) + plant extract (400mg/kg), p.o daily. (Figure 5) |

**BLOOD COLLECTION**

After end of treatment period, the animals were anaesthetized with ketamine 2mg/kg (i.p route), blood was collected by Retro orbital puncture , with EDTA and 7 without EDTA for the enumeration of blood cell (i.e. RBC, WBC,),estimation of Haemoglobin and for estimation of various biochemical parameters respectively.

**SEPARATION OF SERUM**

For estimating the biochemical parameters such as SGOT, SGPT, ALP, TOTAL PROTEIN, serum was separated from blood by centrifuging at 10,000 rpm for 10 minutes. The separated serum were collected and used.) for the parameter estimation. [6]

**SEPARATION OF PLASMA**

For the estimation of tumour markers such as Alpha-feto-protein (AFP), Carcinoembroyonic antigen (CEA), the blood was collected with EDTA, and centrifuged at 10,000 rpm for 5 min. the separated plasma was used for the parameter estimation.

**ESTIMATION OF HEMATOLOGICAL PARAMETERS**

**ESTIMATION OF RBC**

**PROCEDURE**

The RBC pipette was filled with blood up to the mark 0.5, immediately RBC diluting fluid (Hayem's fluid) was filled up to the mark 101. Pipette was rolled between the palms to ensure thorough mixing of blood with diluting fluid and kept aside for a while. The counting chamber was placed and the RBC squares were focused under low power first, when markings were identified then turn to high power. The first 3-4 drops of blood mixture was discarded and it was mixed once again, the counting chamber was charged with the mixed blood. After charging mount the slide, allow the fluid to settle then using a 45X lens the RBC

were counted uniformly in corner and middle squares. The number of cells were expressed as $10^{12}$/ Cmm.[7]

Results were Tabulated in Table 3.

**ESTIMATION OF WBC**

**PROCEDURE**

The WBC pipette was filled with blood up to the mark 0.5, immediately RBC diluting fluid (Hayem's fluid) was filled up to the mark 11.Pipette was rolled between the palms to ensure thorough mixing of blood with diluting fluid and kept aside for a while. The counting chamber was placed and the WBC squares were focused under low power first, when markings were identified then turn to high power .The first 3-4 drops of blood mixture was discarded and it was mixed once again, the counting chamber was charged with the mixed blood. After charging mount the slide, allow the fluid to settle then using a 10X lens the WBC were counted uniformly in corner squares. The number of cells was expressed as 10[9] Cmm.[7]

Results were Tabulated in Table 3.

**ESTIMATION OF HEAMOGLOBIN**

**PROCEDURE**

The heamoglobinometer tube was filled with N/ 10 HCl up to the marking 10, to this 20µl of blood was added with the help of pipette. The superficial ac1d was sucked and it rinsed repeatedly till all the blood in the pipette washed out in mud. The contents in the tube were mixed by stirring, and allowed to stand for 10 minutes. A clear brown colour solution was formed due to the formation of acid hematin. Then distilled water was added drop by drop to dilute. The colour of diluted fluid was compared with the standard; dilution was continued until the colour of the flu1d exactly matches the standard. The lower meniscus of the fluid was noted and reading was noted directly 31 ~ from the graduated tube as g/ 100ml or as percentage of heamoglobin.[7]

Results were Tabulated in Table 3.

**ESTMATION OF SERUM BIOCHEMICAL PARAMETERS**

**Estimation of Serum glutamate oxalo acetate transaminase (SGOT)**

**Method:** Optimized UV- test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

**Assay procedure**

a. To 800 µ1 of reagent-1, 200 µ1 of reagent-2 was added and mixed in a test tube.
b. To the above mixed reagents, 100 µ1 of serum was added.

It was mixed and the readings were taken immediately in a semiautoanalyser. (Model-Photometer5010).

The level of SGOT was expressed as Units/liter [8]
Results were Tabulated in Table 4.

**Estimation of Serum glutamate pyruvate transaminase (SGPT)**

**Method:** Kinetic U V test, according to the international federation of clinical chemistry and laboratory medicine (IFCC)

**Assay procedure:** Mix 800 µ1 of reagent-l with 200 µ1 of reagent-2 in a 5 ml test tube.
a) To this, added 100 µl of serum.
b) Mixed well and took the reading immediately.

Normal range: < 41 u/1
The level of SGPT was expressed as Units/Liter [8]
Results were Tabulated in Table 4.

**Estimation of Total cholesterol (TC)**

**Method**

CHOD-PAP: enzymatic photometric test

**Assay procedure**

a. 1 ml (1000 µl) of reagent-1 has taken in a 5 ml test tube.
b. Added 0.01 ml (10 µl) of serum.
c. Mixed well and incubated at $37^0$C for 5 min.
d. Read the test sample.

Normal Range: < 200 mg/dl in serum.

The level of Total Cholesterol was expressed as mg/dl. [9]
Results were Tabulated in Table 4.

**Triglycerides (TG)**

**Method**

Colorimetric enzymatic test using glycerol-3-phosphate-oxidase (GPO).

**Assay procedure**

a. 1 m1 (1000 µl) of reagent-l has taken in a 5 ml test tube.

b. Added 0.01 ml (10 µl) of serum.

c. Mixed well and incubated at 37°C for 15 min.

d. Read the test sample.

**Normal range**: < 200 mg/dl in serum. The level of Triglycerides was expressed as mg/dl. [9] Results were Tabulated in Table 4.

**ESTIMATION OF TUMOR MARKERS**

**Carcinoembroyonic antigen (CEA)**

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent to each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (I8-25°C) for 60 minutes.
6. Remove the incubation mixture by emptying plate content into a waste container.
7. Rinse and empty the microtiter wells 5 times with distilled or deionized water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue colour changes to yellow colour completely.
13. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes. [10] Results were Tabulated in Table 5.

**Alpha-fetoprotein (AFP)**

**Assay Procedure**

1. All reagents should be brought to room temperature (18-25 $^{o}$C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8 °C
3. Secure the desired number of coated wells in the holder.
4. Dispense 20 µl of standard, specimens, and controls into appropriate wells.
5. Dispense 100 µ1 of Zero Buffer into each well.
6. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
7. Incubate at room temperature (18-25 $^{o}$C) for 30 minutes.
8. Remove the incubation mixture by flicking plate content into a waste container.
9. Rinse and flick the microtiter wells 5 times with distilled or deionized water.
10. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
11. Dispense 150 pl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
12. Incubate at room temperature for 30 minutes.
13. Remove the incubation mixture by flicking plate contents into a waste container.
14. Rinse and flick the microtiter wells 5 times with distilled or deionized water.
15. Strike the wells sharply onto absorbent paper to remove residual water droplets.
16. Dispense 100 pl TMB Reagent into each well. Gentle mix for 10 seconds.
17. Incubate at room temperature for 20 minutes.
18. Stop the reaction by adding 100 µl of Stop Solution to each well.
19. Gently mix for 30 seconds. It is important to make sure that all the blue colour changes to yellow colour completely.
20. Read optical density at 450 nm with a microtiter reader within 15 minutes. [11]

Results were Tabulated in Table 6.

**Collection of tissues:** After the blood collection the animals sacrificed and the body was cut opened, and gross pathological changes were observed and the organs like liver, kidney, and colon were excised immediately and washed with normal saline and wet organ weight was

determined, Portion of the was preserved in 10% buffered neutral formalin solution for histopathalogical study and from the remaining portion of organ, was subjected to tissue homogenate preparation.

Results were Tabulated in Table 7.

**ENZYMIC ANTIOXIDANTS ASSAYS**

**Estimation of Proteins:** Protein was estimated by the method of Lowry *et al*., (1951).

**PRINCIPLE**

**This method is a combination of both Folin-ciocalteau and Biuret reaction which involves two steps**

Step: l-Protein binds with copper in alkaline medium and reduces it to $Cu^{2+}$.

Step2-The cu++ formed catalyses the oxidation reaction of aromatic amino acid by reducing Phosphomolybdotungstate to heteropolymolybdanum, which leads to the formation of blue colour and absorbance was measured at 640nm.[12]

Results were Tabulated in Table 8.

**Estimation Superoxide dismutase [SOD, EC 1.15.1.1]**

SOD was assayed by the method of Kakkar *et al.*, (1984).

**Procedure**

0.5 ml of the tissue homogenate (homogenized in 0.052 M sodium pyrophosphate buffer pH 8.3) or 0.5 ml erythrocyte lysate was diluted to 1.0 ml with ice-cold water followed by the addition of 2.5 ml ethanol and 1.5 ml chloroform (chilled reagents), shaken for 90 sec at 4°C and then centrifuged. The enzyme activity in the supernatant was determined as follows: The assay mixture contained 1.2 ml sodium pyrophosphate buffer, 0.1 ml PMS, 0.3 m1 NBT and appropriately diluted enzyme preparation in a total volume of 3.0 ml. The reaction was started by the addition of 0.2m1 NADH. After incubation at 30°C for 90 see, the reaction was stopped by the addition of 1.0 ml glacial acetic acid. The reaction mixture was stirred vigorously, shaken with 4.0 m1 n-butanol and was allowed to stand for 10 min. After centrifugation the colour intensity of the chromogen in butanol layer was measured in a colorimeter at 520 nm. A system devoid of enzyme served as control.

The enzyme concentration required to produce 50% inhibition of chromogen formation in one minute under standard condition was taken as one unit. The specific activity of the enzyme is expressed as enzyme required for 50% inhibition of NBT reduction/min/mg Hb for erythrocyte lysate and enzyme required for 50% inhibition of NBT reduction/min/mg protein for tissues.

The level of SOD was expressed as units/min/mg protein.[13]

Results were Tabulated in Table 8.

**Estimation of Catalase [CAT, EC 1.11.1.6]**

The activity of CAT was determined in erythrocyte lysate and tissue homogenate by the method of Sinha (1972).

***Procedure***

Tissue homogenate was prepared in phosphate buffer. To 0.9 ml of phosphate buffer, 0.1 m1 erythrocyte lysate or tissue homogenate and 0.4 ml $H_20_2$ were added. The reaction was arrested after 15, 30, 45 and 60 sec by adding 2.0 ml dichromate- acetic acid mixture. The tubes were kept in a boiling water bath for 10 min, cooled and the colour developed was read at 590 nm. Standards in the concentration range of 20-100 ump were processed as for test.

The specific activity of the enzyme is expressed **as µmoles of $H_20_2$ utilized/min/mg Hb** for erythrocyte lysate and **µmoles of $H_20_2$ utilized/min/mg protein** for tissues.[14]

Results were Tabulated in Table 8.

**Glutathione peroxidase [GPX, EC 1.11.1.9]**

The activity of GPx was assayed in erythrocyte lysate and tissue homogenate by the method of Rotruck *et al.*,. (1973).

**Procedure**

0.2 ml of Tris-HCL buffer, 0.2 ml EDTA, 0.1 ml sodium azide and 0.2 ml enzyme preparation (erythrocyte lysate or tissue homogenate) were added and mixed well. To this, 0.2 ml GSH followed by 0.1 ml H2O2 were added. The contents were mixed and incubated at 37 c for 10 min. The reaction was arrested by the addition of 0.5 ml TCA. The tubes were centrifuged and the remaining GSH was determined colorimetrically at 340 nm.

The activities are expressed as µmoles of GSH utilized/min/mg. Hb for erythrocyte lysate and µmoles of GSH utilized/min/mg protein for tissues.[15]

Results were Tabulated in Table 8.

**Estimation of Reduced glutathione (GSH)**

GSH in erythrocyte lysate and tissues were estimated by the method of Boyne and Ellman (1972).

**Procedure**

1.0 ml of erythrocyte lysate or tissue homogenate was precipitated with 2.0 ml TCA and centrifuged. To 1.0 ml of the supernatant, 3.0 ml phosphate buffer and 0.5 ml Ellman`s reagent were added. The yellow colour developed was read in a colorimeter at 412 nm. A series of standards (20-100 g) were treated in a similar manner along with a blank containing 1.0 ml buffer. The amount of GSH is expressed as ug/dl erythrocyte lysate and mmoles/mg tissue. [16]

Results were Tabulated in Table 8.

**ESTIMATION OF GLUCONEOGENIC ENZYMES**

Isolation of mitochondria and microsomes :The mitochondria of liver and kidney were isolated by the method of *Johnson and Lardy (1967)* and microsomes by Hanioka *et al.*, (1997).

A 10% (w/v) homogenate was prepared in 0.05 M Tris-HCL buffer, pH 7.4 containing 0.25 M sucrose and centrifuged at 600* g for 10 min. The supernatant fraction was decanted and centrifuged at 15,000 * g for 5 min. The resultant mitochondrial pellet was then washed and resuspended in the same buffer.

The post mitochondrial fraction was further centrifuged at 105,000 * g for 60 min. The microsomal pellet was suspended in 0.05 M Tris-HCL buffer, pH 7.5 containing 0.15 M KCL.

The purity of mitochondrial and microsomal fractions was assessed by measuring the activities of succinate dehydrogenase and glucose-6-phosphate dehydrogenase respectively.[17]

Results were Tabulated in Table 8.

**Evaluation of colon cancer by aberrant crypt foci:** After the completion of 16 weeks treatment all the animals are sacrificed and collect the colons of all the rats. Cut the colons longitudinally, to expose the luminal surface. Flush with potassium phosphate buffer. The opened colons are placed between the filter papers and placed in 10% formalin fixative overnight, and then place the 2cm long segments in a petridish and stained with 0.2% methylene blue solution. And total number of aberrant crypt per focus was counted.

Results were Tabulated in Table 7.

**Extraction and determination of fecal neutral sterols**

Extraction : From the collected fecal matter, 0.5 g is suspended in 4.0 mL distilled water and is hydrolyzedat 80ºC in 4.0 mL of 2 N methanolic NaOH for 2 h. To the residue, 4.0 mL of 2 N methanol is added and the neutral sterols was extracted by adding 5.0 mL of petroleum ether (60-80ºC).

Determination: Neutral sterols was estimated in feces by the method of Miettinen *et al*., (1965).

**R**esults were tabulated in table 9.

**Extraction and determination of fecal bile acids**

Extraction: During the period of study, 24 h stool samples was collected every week and also before sacrifice of the rats in all groups. The stool samples was weighed, homogenized with equal amounts of water (w/v) and dried at 110ºC for 24 h. After drying and reweighing, the feces was lyophilized to a fine powder. and 1.0 mL KOH in ethylene glycol is added. The mixture is heated at 220ºC for 15 min with occasional mixing. After cooling, 1.0 mL NaCl, 0.2 mL conc. HCl and 6.0 mL diethyl ether was added to the acidified solution. The tube is shaken for 1 min and centrifuged at 2000 ´g for 3 min; the upper layer is collected, evaporated at 40ºC, the residue is dissolved in 1.0 mL methanol and used for the determination of bile acids. Determination : Bile acids was estimated in feces by the method of de Wael *et al*., (1977).[18]

Results were tabulated in table 9.

## HISTOPATHOLOGY

The organs/ tissues are collected and washed under saline and preserved in 10% buffered neutral formalin (DNF) the trimmed tissue sections are subjected to prepare paraffin blocked. 5 micron thickness sections were cut and stained with Haematoxylin and Eosin (H&E) and observed under compound microscope.[19] Figure (6,7,8,9,10,11,12,13,14 and 15).

### Statistical analysis

Data collected from the above specified studies were subjected to One-way ANOVA followed by Dunnet`s comparison by using Graph pad prism 5. Version 5.01.

The significance was expressed as ***P<0.001, **P<0.01, *P<0.05, #- Non Significant.

## RESULTS

**Table 1: Percentage Yeild of Extract**

| S.No | Extracts | NATURE OF EXTRACT | COLOUR | WEIGHT (GM) | PERCENTAGE YEILD (%) |
|---|---|---|---|---|---|
| 01 | Aqueous | SEMI-SOLID | Dark brown | 280.19 | 28.1 |

### *IN VIVO* ANTI CANCER SCREENING ON DMH INDUCED COLON CANCER

**Fig:1, GROUP – I: CONTROL - Normal saline. Fig:2, GROUP – II Only DMH**

**Fig:3, GROUP – III: DMH + 5 Fluorouracil Fig:4, GROUP – IV: Plant ext low dose + DMH**

**Fig:5, GROUP: V – Plant ext high dose + DMH**

**Table 2: Determination of Animals Body Weight**

| Weeks | Normal | DMH | DMH+STD | Extract 200mg | Extract 400mg |
|---|---|---|---|---|---|
| 1 Week | 337±1.8 | 330±2.4 | 345.3±1.4 | 340.08±1.8 | 342.12±1.7 |
| 3 Week | 395±2.0 | 379.8±1.6 | 388.4±1.3 | 364.85±1.1 | 376.18±1.4 |
| 5 Week | 460±1.2 | 420.6±2.1 | 434.9±2.4 | 393.05±2.5 | 412.11±2.2 |
| 7 Week | 518±1.6 | 456.2±1.8 | 476.1±2.0 | 419.65±2.5 | 450.96±1.1 |
| 9 Week | 574±2.2 | 483.1±1.1 | 517.3±1.1 | 448.16±2.0 | 488.84±1.9 |
| 11 Week | 634±2.0 | 500.7±2.1 | 563.5±1.8 | 476.79±1.1 | 526.86±2.5 |
| 13 Week | 690±1.8 | 509.7±2.2 | 609.5±2.2 | 504.49±1.9 | 565.11±2.0 |
| 15 Week | 760±1.9 | 511.8±1.4 | 656.2±1.0 | 541.12±1.7 | 608.84±1.8 |

**Table 3: ESTIMATION OF HEMATOLOGICAL PARAMETERS.**

| GROUPS (n=6) | CONTROL Group - I | Only DMH Group - II | DMH + 5 Fluorouracil Group - III | DMH+ AEPQ (200mg/kg) Group – IV | DMH+ AEPQ (400mg/kg) Group – V |
|---|---|---|---|---|---|
| RBC ( 1 x $10^{12}$/L ) | 5.1866+0.0882 | 4.5366+0.0962** | 5.92333+0.1337*** | 4.220+0.1149*** | 5.400+0.1299ns |
| WBC ( 1 x $10^{19}$/L ) | 12.43+0.3106 | 14.73+0.2765*** | 11.27+0.1725ns | 15.17+0.3691*** | 11.87+0.5869ns |
| Hb ( g/dL ) | 12.533+0.2564 | 10.766+0.4005* | 15.066+0.532*** | 9.666+0.3392*** | 13.3000+0.4427ns |
| Lymphocytes % | 80.00+1.932 | 82.666+1.1737 ns | 83.666+2.8362ns | 87.333+1.282ns | 85.666+2.740ns |
| Monocytes % | 3.333+0.5678 | 3.333+0.4216ns | 4.000+0.3651ns | 3.667+0.7601ns | 3.33+0.5578ns |
| Eosinophils % | 4.333+0.760 | 5.000+0.3651ns | 4.333+0.7601ns | 5.666+0.4216ns | 3.666+2108ns |

***P<0.001, **P<0.01, *P<0.05, #-Non significant

Data is expressed as Mean +SEM. ( n=6, animals in each group )

**Table 4: Estimation of Serum Biochemical Parameters**

| GROUPS (n=6) | CONTROL Group – I | Only DMH Group –I I | DMH+5 FLUOROURACIL Group – III | DMH+AEPQ (200mg/kg) Group – IV | DMH+AEPQ (400mg/kg) Group – V |
|---|---|---|---|---|---|
| **SGOT (U/L)** | 125.0+4.359 | 181.7+1.453** | 130.0+5.168* | 140.0+8.021** | 142.0+0.5774** |
| **SGPT (U/L)** | 66.33+2.404 | 86.67+2.333*** | 68.34+3.510* | 62.67+2.963ns | 64.67+4.256ns |
| **Total cholesterol (mg/dl)** | 64.00+4.041 | 115.0+3.215*** | 84.00+5.041** | 99.33+2.186*** | 92.67+2.333** |
| **Triglycerides (mg/dl)** | 94.17+3.491 | 56.13+8.781ns | 98.2+4.356* | 120.1+4.477** | 103.6+14.47*** |
| **Total Bilirubin (mg/dl)** | 0.6667+0.03333 | 0.6667+0.1202 | 0.1568+0.0ns | 0.2000+0.05774ns | 0.1667+0.03333ns |

***P<0.001, **P<0.01, *P<0.05, #-Non significant

Data is expressed as Mean +SEM. ( n=6, animals in each group )

**Table 5: Estimation Of Carcinoabroyonic Antigen (Cea)**

| GROUPS (n=6) | CONTROL Group – I | Only DMH Group –I I | DMH+5 Fluorouracil Group – III | DMH+AEPQ (200mg/kg) Group – IV | DMH+AEPQ (400mg/kg) Group - V |
|---|---|---|---|---|---|
| **Carcinoembroyonic antigen (ng/dL)** | 0.1953+0.00206 | 0.4733+0.007126* | 0.2111+0.003000*** | 0.2660+ 0.006083*** | 0.2377+0.002404*** |

***P<0.001, **P<0.01, *P<0.05, #-Non significant

Data is expressed as Mean +SEM. (n=6, animals in each group )

**Table 6: Estimation of Alpha-Feto-Protein (AFP)**

| GROUPS (n=6) | CONTROL Group – I | Only DMH Group –I I | DMH+5 Fluorouracil Group – III | DMH+AEPQ (200mg/kg) Group – IV | DMH+AEPQ (400mg/kg) Group - V |
|---|---|---|---|---|---|
| AFP (ng/dL) | 0.4800+0.01528 | 0.6780+0.007234* | 0.5000+04947*** | 0.6000+ 0.005773*** | 0.5433+0.01856*** |

***P<0.001, **P<0.01, *P<0.05, #-Non significant

Data is expressed as Mean +SEM. ( n=6, animals in each group )

**Table 7: Effect of aqueous extract of *Portulaca quardifida* (AEPQ) on tumor volume, burden, weight and tumor incidence analysis (physical parameter) on DMH induced colorectal cancer in rats**

| Group | Tumor Volume | Tumor Burden | Tumor Incidence | Colon Weight gms |
|---|---|---|---|---|
| Normal | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.76±0.02 |
| Control | 4.55±0.67*** | 13.17±2.04*** | 22.83±3.81*** | 1.27±0.18*** |
| Standard | 1.96±0.41*** | 4.50±1.87*** | 9.83±1.72*** | 0.86±0.12*** |
| Test I | 2.99±0.59*** | 6.16±1.16*** | 15.50±1.87*** | 1.11±0.06ns |
| Test II | 2.20±0.29*** | 5.83±1.47*** | 13.33±1.63*** | 0.92±0.22** |

**Table 8: Invivo Antioxidant Activity In Colon Tissue**

| GROUPS (n=6) | CONTROL Group – I | Only DMH Group - II | DMH+5 FLUOROURACIL Group – III | DMH+AEPQ (200mg/kg) Group – IV | DMH+AEPQ (400mg/kg) Group – V |
|---|---|---|---|---|---|
| PROTEIN Mg/GTissue | 0.4900+0.02309 | 0.2390+0.02610ns | 0.4319+0.0611ns | 0.4040+0.007024ns | 0.4117+0.01922 ns |
| SOD (Units/min/mg/protein) | 5.460+0.2272 | 2.827+0.08090ns | 4.009+0.01298ns | 3.380+0.02646ns | 3.930+0.09849ns |
| CATALASE µ/moles of H2O2 consumed / min / mg protein | 41.60+0.8630 | 28.46+0.7681 ns | 29.82+0.1412 ns | 24.40+0.2797 ns | 28.41+0.7664 ns |
| GPx µ/moles protein | 70.2+1.10 | 51.4+0.60ns | 64.2+0.80ns | 59.6+0.90* | 68.43+1.01ns |
| GSH Gulatathione µ/moles | 87.6+3.105 | 59.97+3.73ns | 91.20+229** | 77.11+8.23ns | 86.6+1159ns |
| LPx MDA formed/Mg protein | 70.1+2.115 | 103.2+3.822 | 86.11+2.985*** | 78.60+1.148*** | 65.15+1.875*** |

***P<0.001, **P<0.01, *P<0.05, ns-Non significant

Data is expressed as Mean +SEM. ( n=6, animals in each group )

**Table 9: Determination of Faecal Neutral Sterols, Faecal Bile Acids and Dried Faecal Weight**

| GROUP | CONTROL | ONLY DMH | DMH + STD | DMH + LOW DOSE | DMH + HIGH DOSE |
|---|---|---|---|---|---|
| Dried faecal weight (g) | 0.1040±0.046 | 0.0710±0.0317 | 0.0945±0.0423 | 0.1135±0.0507 | 0.097±0.0434 |
| Faecal bile acid excretion (mg) | 0.0110±0.0050 | 0.0223±0.0104 | 0.0137±0.0066 | 0.0127±0.0057 | 0.0113±0.0050 |
| Faecal neutral sterol excretion (mg) | 0.5642±0.2523 | 0.8547±0.4042 | 0.5613±0.2510 | 0.6132±0.2749 | 0.5623±0.2515 |

a . Data are expressed as mean ± SEM.

b. Weight gain.

c . Significantly different from control, $p<0.05$.

Group-I CONTROL (COLON)

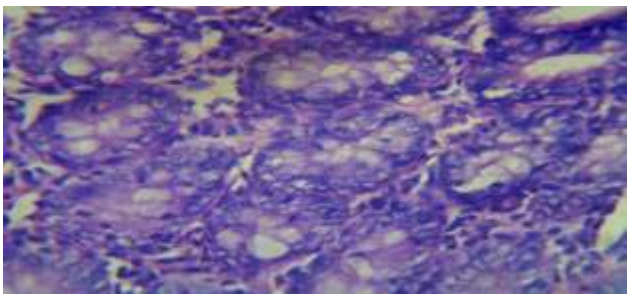

**Figure: 6: 40x shows mild inflammation**

**MICROSCOPIC APPEARANCY**

Section studied from the colon shows normal epithelium. The lamina propria shows scattered lymphocytic infiltration. Muscular layer and serosa shows no significant pathology. There is no evidence of dysplasia/malignancy in the section studied.

Group-I CONTROL (LIVER)

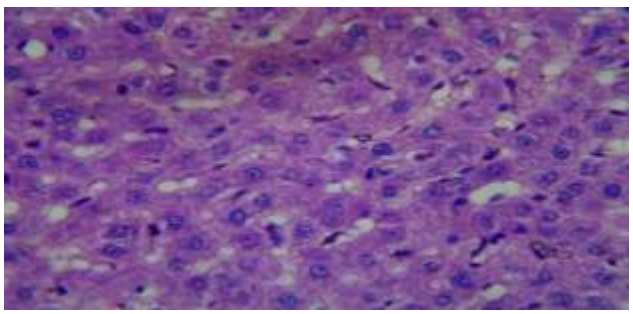

**Figure: 7 40x shows normal portal tract**

**MICROSCOPIC APPEARANCY**

Section studied from the liver shows maintain lobular architecture. Individual hepatocytes show no significant pathology. The central vein shows dilatation and congestion. The portal triad shows bile duct hyperplasia. The sinusoids are dilated.

Group-II ONLY DMH ( COLON)

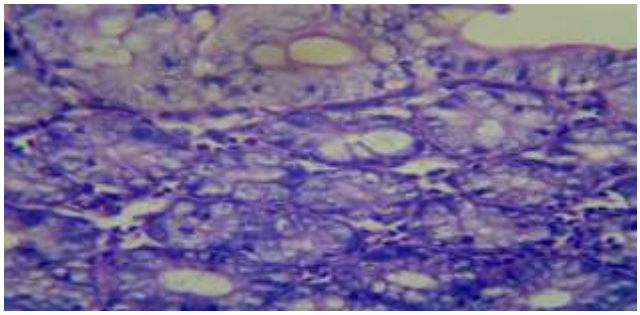

**Figure: 8 40x shows individual cells increase in number of stratification**

**MICROSCOPIC APPEARANCY**

Section studied from the colon shows increased number of glands with stratification. Individual cells are round to oval with moderate eosinophilc cytoplasm and vesicular nuclei showing mild dysplasia with prominent nucleoli. Occasional mitosis are also seen.

Group-II ONLY DMH (LIVER)

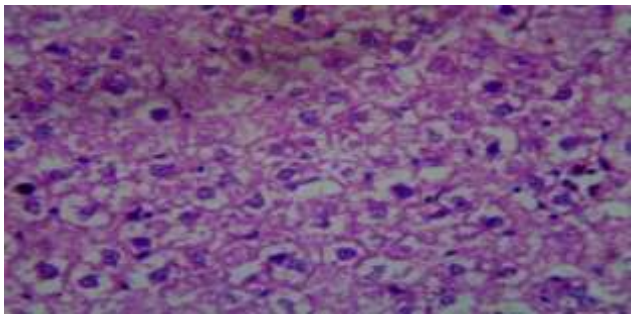

**Figure: 9 40x shows cytoplasmic vacuolation**

**MICROSCOPIC APPEARANCY**

Section studied from the liver shows distorted architecture with mild parenchymal inflammation. Individual hepatocytes show cytoplasmic vacuolation.The central vein shows normal. The portal triad shows lymphocytic infiltration. The sinusoids show no significant pathology.

Group-III DMH + STD (COLON)

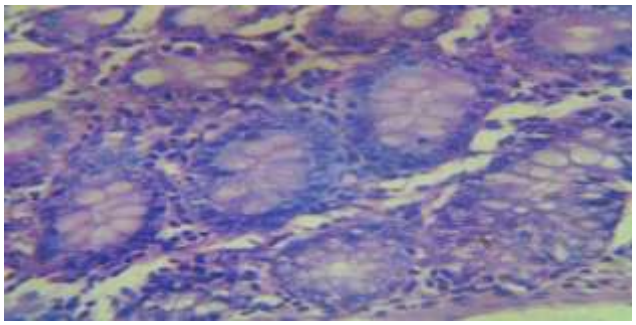

**Figure: 10 40x shows normal crypts**

**MICROSCOPIC APPEARANCY**

Section studied from the colon shows normal mucosal epithelium. The lamina propria shows lymphocytic infiltration. Muscular layer and serosa shows no significant pathology. There is no evidence of dysplasia/malignancy in the section studied.

Group- III DMH + STD ( LIVER)

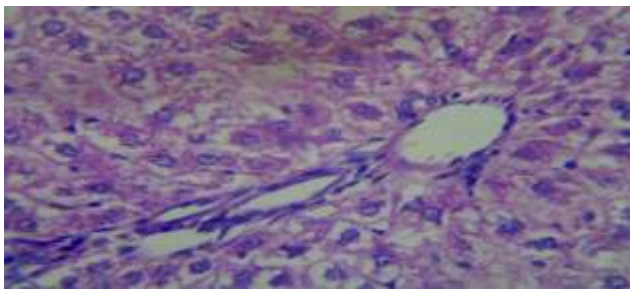

**Figure: 11: 40x shows central vein normal sinusoidal dilatation**

**MICROSCOPIC APPEARANCY**

Section studied from the liver shows mild distorted architecture. Individual hepatocytes show cytoplasmic vacuolation. The central vein shows normal. Sinusoids show mild dilatation. The portal triad shows diffuse scattered lymphocytic infiltration.

Group-IV DMH + LOW DOSE (COLON)

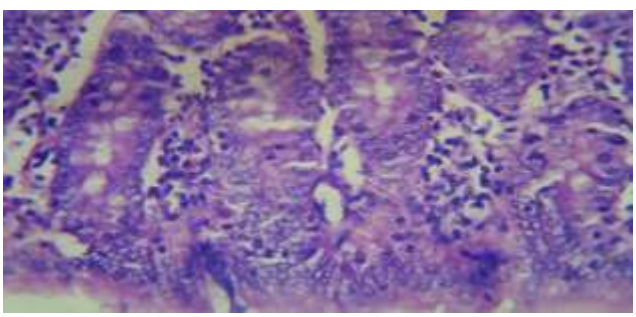

**Figure: 12 40x shows inflammation**

**MICROSCOPIC APPEARANCY**

Section studied from the colon shows normal epithelium increased number of glands with stratification are also seen. Individual cells are round to oval with moderate eosinophilc cytoplasm and vesicular nuclei showing mild dysplasia with prominent nucleoli. Occasional mitosis are also seen. The lamina propria shows scattered lymphocytic infiltration.

Group- IV DMH + LOW DOSE (LIVER)

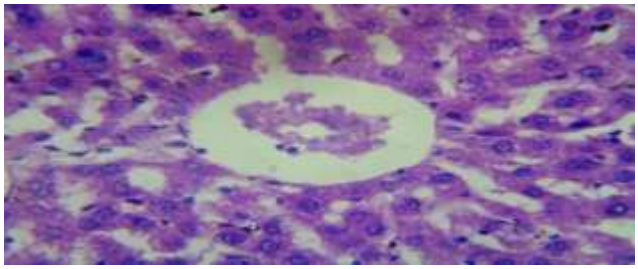

**Figure: 13 40x shows central vein normal and sinusoidal dilatation**

**MICROSCOPIC APPEARANCY**

Section studied from the liver shows distorted architecture. Individual hepatocytes show focal hepatocytic necrosis. The central vein shows normal. The portal triad shows dense lymphocytic infiltration.

Group-V DMH + HIGH DOSE. ( COLON)

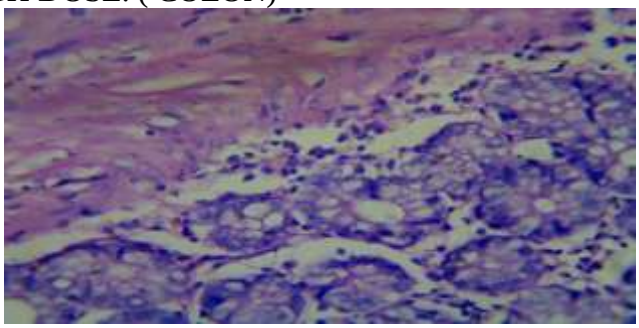

**Figure: 14: 40x shows normal colon with mild inflammation**

**MICROSCOPIC APPEARANCY**

Section studied from the colon shows normal epithelium. The lamina propria shows mild lymphocytic infiltration. Muscular layer and serosa shows normal. There is no evidence of dysplasia/malignancy in the section studied.

Group- V DMH + HIGH DOSE ( LIVER)

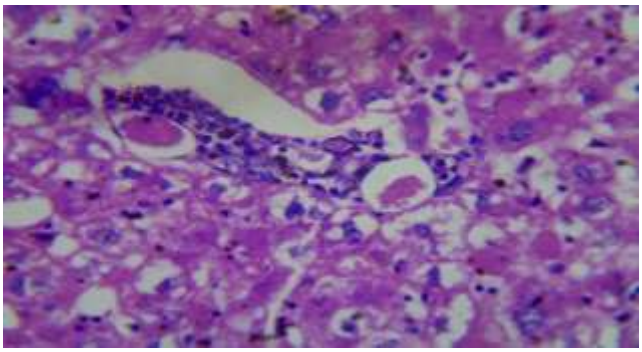

**Figure: 15: 40x shows portal tract inflammation with cytoplasmic vacuolation**

## MICROSCOPIC APPEARANCY

Section studied from the liver shows lobular architecture. Individual hepatocytes show cytoplasmic vacuolation. The central vein shows dilatation. Sinusoids show mild dilatation. The portal triad shows lymphocytic infiltration.

## DISCUSSION

However space does not permit a long approachable discussion to improve efficiency in the context of anticancer activity.

**In summary, the key areas on which our work is based are as follows**

- **Hematological parameters:** The extract reversed the abnormal values of WBC, RBC, blood cell count to normal values.
- **Tumor markers:** The extract decreased the levels of CEA and AFP which indicates positive prognosis.
- **Enzymic and non enzymic antioxidants:** The extract brings about profound alterations in the tissue lipid per oxidation and anti oxidant status.
- **Aberrant crypt foci (ACF):** The extract treated group shown significant reduction in the formation of ACF.
- **Histopathology:** The extract treated group does not showed any abnormality when compared with DMH treated group

The above summary clearly indicates that the aqueous extract of *Portulaca quadrifida* possesses good anticolorectal activity.

## CONCLUSION

When traditional medicine is blended into holistic synergy it tunes to provide affordable health care related to cancer for every citizen of global human society.

In conclusion, our study suggest that aqueous extract of *Portulaca quadrifida* exerts its anti colorectal cancer activity, before its clinical usage, through toxicological profile.

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